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APPLICATION OF HIGH DOSE RADIATION
TO
PANCREATIC TRANSPLANTATION

by

© Richard W. Nason

A THESIS

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Supervisor

Date June 28, 1985

ABSTRACT

An efficient means of separating the endocrine tissue from other components of the pancreas is necessary to bring islet cell transplantation closer to clinical trials. Based on observations that islet tissue is less radiosensitive than other components of the pancreas high doses of radiation were used to purify a graft preparation of pancreatic microfragments.

The graft (n=8), prepared by enzymatic digestion and mechanical disruption was divided into 4 groups. Group I served as a control and the remaining groups were irradiated: II - 2500 rad; III - 5000 rad; IV = 7500 rad. Purification was assessed by determining the ratio of insulin to amylase (I/A) per g of tissue in the pancreas (P;n=7), untreated graft (G;n=7) and experimental groups (I - IV) immediately after radiation (n=8) and 24 h (n=6) of tissue culture. Following radiation structural changes in exocrine tissue were evaluated with electron microscopy (EM) and viability of endocrine cells was assessed with invitro glucose challenge in perifusion. Islet recovery and viability in a graft treated with 5000 rad was assessed invivo by comparing survival and metabolic function (ivGTT) at 1 mo postimplant in 9 dogs receiving an untreated graft (group Ia) to 7 dogs receiving the irradiated graft (group IIa).

The I/A ratio increased with radiation: P = 0.66± 0.13; G = 0.18± 0.08; I = 2.86± 1.09; II = 2.64± 1.11; III = 5.24± 2.30; IV = 4.64± 1.86. The I/A ratios of groups III and IV differed significantly ($P < 0.05$) from the I/A ratio of the untreated graft. The increase in the I/A ratio with radiation was not associated with a significant decrease in amylase content (IU/g). The insulin content (uU/g in thousands) increased (NS) with radiation: P=1.47± 0.41; G=1.12± 0.27; I=1.59± 0.48 ; II=1.54±0.38; III=2.11± 0.43; IV=2.15± 0.53. The I/A ratio measured after 24 h of tissue culture had increased (NS) in groups I - IV: I=6.68± 0.31; II=4.33± 0.19; III=6.33± 0.32; IV=3.68± 0.14. The trends observed in culture were similar in groups I - IV. The amylase content (IU/g) was significantly ($P < 0.05$) decreased by 8 h and after 24 h of culture the insulin content (IU/g) had decreased to less than 50% of the original levels. The only significant ($P<0.05$) difference in groups I - IV was the decrease in insulin content (uU/g) in group IV from 8 h to 24 h of culture. EM showed progressive injury in irradiated exocrine cells (degranulation, vacuoles in cytoplasm, nuclear changes). A biphasic release of insulin, similar in groups I - IV, was observed in perifusion. The survival of technically successful autografts 1 mo postimplant was comparable in both groups: Ia=6 of 7 dogs; IIa= 5 of 7 dogs. Metabolic function (ivGTT) was significantly better ($P<.001$) in group Ia as reflected by percent decline in glucose (K value): Ia=1.66± 0.25; IIa=0.93± 0.16, and peak insulin (uU/ml): Ia=7.2± 0.9; IIa=2.0± 0.7.

Radiation in high doses is a simple and efficient means of purifying islet cell grafts. The recovery of viable islets after radiation permits better autograft survival than previously reported after other means of purifying islet cell grafts in the canine model.

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I INTRODUCTION

Within six months of the report of the successful isolation of insulin from the islets of Langerhans by Banting and Best in 1921 the injection of insulin was applied to the clinical therapy of diabetes. It soon became apparent however that although insulin therapy prevented many of the acute metabolic problems such as ketoacidosis, other sequelae, glomerulopathy,retinopathy, and neuropathy, appeared in most patients with the insulin dependent form of the disease for two or more decades. These complications had been noted prior to the advent of insulin but were relatively unusual because death from ketoacidosis or infection shortened the life of the patient before they could become manifest. Thus insulin, although a tremendous step forward, did not provide the total solution for the diabetic patient. (1)

Evidence that precise regulation of blood glucose will defer, prevent or even reverse early diabetic microangiopathy is steadily mounting. (2, 3,4) However until a treatment exists that will normalize blood glucose levels during activities of daily living, the controversy concerning cause and effect of hyperglycemia and complications will not be entirely resolved. (4)

Advances in the administration of exogenous insulin such as multiple daily insulin injections, home glucose monitoring and insulin infusion pumps have been developed in an attempt to maintain nearly constant normoglycemia. Their effectiveness in preventing, stabilizing or reversing the vascular complications of diabetes has not been determined. (5,6)

The complexities involved in the neuroendocrine modulation of islet cell secretion are just being recognized. (7,8) It does not seem likely that attempts to mimic this intricate control mechanism by the administration of one hormone, insulin, will provide the degree of glucose control necessary to prevent the complications of diabetes. (9, 10)

Total endocrine replacement therapy in the form of transplantation of the intact pancreas, the distal segment of the pancreas, or the islets themselves can experimentally at least, halt or even reverse the microvascular complications of diabetes. (9) Pancreatic transplantation is the most physiologic approach to the treatment of diabetes. (4,9,11)

The earliest clinical efforts were directed at whole organ transplantation. The results were at best, dismal, with a high incidence of technical failure and severe complications secondary to the exocrine secretion of the pancreas. Recent technical improvements, such as transplantation of the distal segment of the pancreas occluding the duct with synthetic polymer to control exocrine activity, have revived interest in this approach with better patient survival. (11,12,13) The major problems with vascularized pancreatic allografts are deterioration of duct occluded grafts with time secondary to fibrosis and the necessity for recipients to receive generalized immunosuppression. The need for generalized immunosuppression limits the application of whole organ or segmental grafts, at present,to diabetic patients with complications that would otherwise progress to a stage more serious than the potential side effects of antirejection therapy. (12,13)

The devastating complications of early attempts to transplant the intact pancreas associated with the presence of exocrine tissue prompted efforts to transplant isolated islets. From a theoretical point of view transplanting the islets of Langerhans would eliminate the problem of handling the exocrine secretions making the procedure less complicated and safer. There was also hope that islet cells would enjoy the immunoprivileged status of other endocrine tissues. (14,15)

Experimentally islet cell transplantation has been very successful. In rodents,pooled fresh islets from several donors can reverse chemically induced diabetes in recipients of the same strain (16,17) and prevent the development of complications of the disease or reverse those present. (18,19) In dogs autotransplantation of fresh pancreatic fragments can reverse the diabetic state. (20-22)

The vulnerability of isolated islets to rejection was recognized in the earliest experimental allografts. (23,24) The recent experimental work of Lacy (25,26) and Lafferty (27,28) in the field of immunoalteration has renewed enthusiasm in allotransplantation of islet cell grafts. Their work has underscored the importance of donor immune cells or 'passenger leukocytes' in allograft rejection. Lacy has demonstrated that by removing the donor immune cells from a graft preparation of isolated islets in rodents, by a period of tissue culture, allograft survival can be prolonged without systemic immunosuppression. The implication of the above work is that islet cell grafts, as opposed to whole organ or segmental grafts, could be safely transplanted in a diabetic patient shortly after the onset of the disease and prevent the devastating microvascular complications. (10,15) This consideration justifies extensive evaluation of islet cell transplantation.

The technological failure to isolate sufficient quantities of islets from the fibrous and compact human pancreas has been the major barrier to clinical success of islet cell transplantation and has returned interest to transplantation of the intact pancreas. (9,10, 14,15,29) The problem of islet yield has in fact precluded successful attempts to test the theory of immunoalteration in large animal models. (26) The complex techniques to purify graft preparations, such as tissue culture, result in a significant loss of insulin secreting tissue. (26, 29)

A practical, efficient,and clinically applicable means of the separating the endocrine component of the pancreas from contaminating acinar tissue, endothelial elements and immune cells is required to bring pancreatic islet cell transplantation closer to widespread and safe clinical trials. In this study, radiation in moderately high doses was delivered to a graft preparation of pancreatic fragments to selectively injure contaminating exocrine tissue, leave endocrine cells viable, and increase islet cell mass and purity.

II THE RATIONALE FOR PANCREATIC TRANSPLANTATION

Pancreatic transplantation is being investigated as a means to control the devastating vascular complications in insulin dependent diabetes. A cure for diabetes will depend on a more complete understanding of its etiology.

DIABETES

Diabetes is a diagnostic term applied to a syndrome of anatomical and biochemical abnormalities which have in common a distrubance of glucose homeostatis secondary to a deficiency of insulin or its metabolic effects. (30)

Diabetics are generally divided into two categories that are clearly different in terms of pathogenesis, prognosis, etiology and response to treatment regimes. (30,31) Patients who depend on insulin for prevention of ketoacidosis have insulin dependent or Type I diabetes mellitus. This form of diabetes most often develops in childhood or adolescence. Diabetics who do not depend on insulin to ward off ketosis have non-insulin dependent or Type II diabetes mellitus. This form of the disease tends to develop in overweight individuals in middle or late life and constitutes ninety to ninety-five percent of all diabetics.

Recent evidence suggests that insulin dependent diabetes mellitus has an important autoimmune component triggered by a viral infection. (31-33) In support of a role for autoimmune factors are the phenomenon of insulitis or round cell infiltration of the islets, a strong association with certain histocompatibility complex alleles and the identification of circulating islet cell antibodies. (32) The fact that identical twins exhibit less than fifty percent concordance for juvenile onset diabetes despite their identical immuno-genetic make-up suggests that genetic factors are not sufficient for this type of diabetes to develop. (30) Environmental factors, mainly viruses,have been implicated. The most direct evidence for the role of viral infection in the pathogenesis of diabetes has been the recovery of Coxsackie virus from the pancreatic tissue of a child who died with meningoencephalitis and recent onset diabetes. The isolated virus, injected into mice, resulted in beta cell destruction and diabetes. (32)

Noninsulin dependent diabetes mellitus appears to be related to an age related and genetically determined insufficiency of beta cell viability and an inactivity related peripheral unresponsiveness to what insulin in available. (34)

COMPLICATIONS OF DIABETES

The complications of diabetes can be divided into two major categories, short-term and chronic.

The short-term complications include ketoacidosis and hyperosmolar coma as well as numerous other physiologic alterations: increased red cell membrane stiffness, impaired phagocytosis by leukocytes, increased platelet aggregation, increased glomerular permeability and filtration, increased retinal microvascular permeability, lowered levels of high density lipoproteins and decreased nerve conduction velocity. (2,30) The pathogenesis of the above can be attributed to a complete lack of insulin or its effectiveness. The alterations are reversible by correcting the hyperglycemia with insulin. (30)

Of more interest to the field of pancreatic transplantation are the chronic diabetic syndromes. The chronic complications can be divided into three major groups: aberrations involving the large and medium sized blood vessels, macroangiopathy or atherosclerosis; disorders that are unique to diabetes involving arterioles and capillaries, microangiopathy; and a collection of abnormalities, many of which are unique to diabetes, involving for example the nervous system and the skin. (30)

The chronic diabetic syndromes give rise to considerable morbidity and mortality. Cahill and Arky (30) summarize the data from the 1975 report of the National Commission on Diabetes to the United States Congress. The life expectancy of a diabetic is two-thirds that of the general population. In contrast to the general population atherosclerosis occurs at an early age and becomes quite severe. Strokes are twice as frequent, myocardial infarction is two to ten times more frequent and peripheral vascular disease fifty to one hundred times more frequent in diabetics. The capillary abnormalities affect predominantly the kidney and the eye. The diabetic is twenty-five times more prone to blindness and partial loss of vision than is the non-diabetic. One in twenty insulin dependent diabetics become blind. Approximately one-half of insulin dependent diabetics develop renal failure.

The complications secondary to the microangiopathy have received the most attention. They are seen most frequently in insulin dependent diabetics. (12) The renal lesion is the most serious and life threatening of the complications. The typical renal abnormality is a diffuse and/or nodular glomerulosclerosis (Kimmelsteil-Wilson disease) which is characterized by a thickening of the basement membrane in the glomerular capillary loops as well as accumulation of glycoprotein in the mesangial region of the glomerulus. (2,35) Within twenty years of the onset of diabetes clinical and pathological evidence of renal disease may be found in nearly all patients with insulin dependent diabetes mellitus. (35) The eye lesions are characterized by the ophthalmoscopically visible features of microaneurysms, retinal hemorrhages, exudates and new vessel formation. Retinopathy may be the first clinical sign of diabetes but more frequently it is seen several years after the onset of other symptoms and rarely it is totally absent in diabetics of long-standing. (36)

The pathogenesis of the chronic diabetic syndromes is less well defined than that of the acute syndromes. One of the major controversies in the study of diabetes, and yet to be resolved, is whether hyperglycemia or some associated disorder causes or accelerates the development of long-term complications. (2,3,37) The alternate possibility is that the complications are primarily determined by genetic factors independent of hyperglycemia. (38)

RELATION OF DIABETIC CONTROL TO DEVELOPMENT OF MICROVASCULAR COMPLICATIONS

A large body of experimental evidence supports the hypothesis that the metabolic environment of the diabetic causes the complications. (2-4, 18,19,37,39,40,41)

The experimental evidence supports a causal relationship between the renal and eye lesions. For example Lee and coworkers (39) demonstrated that diabetic glomerular changes developed in normal kidneys transplanted to diabetic rats. Conversly, these changes were reversible upon transplantation of kidneys from diabetic rats into normal recipients. Pancreatic islet cell transplantation in inbred diabetic rats effectively returns glucose and insulin levels to normal and rapid regression of the glomerular lesions has been demonstrated. (18,19) Engerman and associates (40) have demonstrated in dogs, made diabetic chemically, that the number of microaneurysms found histologically in the retina appeared to be directly related to the severity of the diabetes. Gray and Watkins (41) demonstrated that new vessel formation and retinal capillary dilatation seen in diabetic rats were absent in animals in which diabetes had been corrected by early pancreatic islet cell transplantation.

Clinically, at the present time, there is no scientific proof demonstrating that good control of diabetes in man has a beneficial effect on the course of diabetic complications. According to Tchobroutsky (3) the definite demonstration that the microvascular complications can be prevented by the normalization of blood glucose concentrations in man cannot be made since normoglycemia cannot be sustained from the onset of the disease with our current methods of treatment. The majority of studies have suggested strongly that the better the control the less the rate and severity of diabetic complications.

The clinical study most frequently cited as supporting at least the relationship between high levels of hyperglycemia and the more frequent or severe complications is that by Pirart. (42) In a prospective study of over four thousand patients they concluded that retinopathy, neuropathy and nephropathy are true complications of diabetes with chronic hyperglycemia as an etiologic factor. An earlier clinical study by Keiding, Root and Marble (43) correlated vascular disease with the degree of control in four hundred and fifty-one diabetics under the age of thirty with diabetes between ten and twenty-six years duration. It was observed that with the increasing duration of diabetes there was an increase in extent and severity of atherosclerotic vascular complications and that these complications were observed less often in diabetics in whom the control was precise. Improved diabetic control with continuous subcutaneous insulin infusion improved renal and retinal function in one study. (44) Eschwege and coworkers (45) reported a slower rate of progression in the number of microaneurysms in insulin dependent diabetics treated by divided insulin injection when compared to patients receiving a single daily insulin injection. The fasting blood sugar was markedly lower in the group that received multiple injections of insulin. Miki and associates (46) as well, have correlated the progression of diabetic retinopathy with poor control of blood sugars. Tchobroutsky (3) in 1978 stated that "in as many as twenty or so prospective studies considered valid by recent reviewers only a few have failed to show any relationship between the incidence of retinopathy and glomerulopathy and the quality of diabetic control".

An attractive hypothesis to the mechanism by which hyperglycemia may cause the chronic complications has been proposed. It is based on the fact that many proteins can be glycosylated nonenzymatically and that the degree of glycosylation correlates directly with the mean level of plasma glucose. (38) It is presumed that the glycosylated peptide causes abnormal structure and function of vascular and other tissue. Recent investigation of the structure, function and biosynthesis of glycosylated hemoglobin has provided a means to objectively assess long-term glucose regulation in the diabetic patient. (47)

CURRENT TREATMENT OF DIABETES - THE ADMINISTRATION OF EXOGENOUS INSULIN

Since the successful isolation of insulin in 1921 the standard treatment of insulin dependent diabetes mellitus has consisted of dietary management and intermittent subcutaneous insulin injection. (1,30) The evidence suggesting that the long-term complications of diabetes are secondary to insulin deficiency and hyperglycemia has prompted research into the best posssible means to achieve perfect control of blood glucose.

Advances have been made in the administration of exogenous insulin in an attempt to maintain nearly constant normoglycemia. These advances include multiple daily injections of insulin, home glucose monitoring and artificial devices for continuous delivery of insulin (artificial beta cell, mechanical pancreas). (2,5-7,48)

Considerable effort has been directed towards the development of an artificial beta cell. Development of such devices has taken two directions. The first has been development of a 'closed loop' system where insulin is metered out in appropriate amounts in response to a minute to minute measure of plasma glucose levels. Such devices are presently available for use clinically. (48) For short periods of time they are capable of completely normalizing blood glucose levels following meals, glucose ingestion, or during physical activity. (2) The system is limited predominantly by the technical problem of developing an implantable glucose sensor. Attachment of the patient to an extracorporeal sensor by an intravenous line is required. Because of this problem, 'open loop' systems or insulin infusion pumps were developed. Insulin delivery is preprogrammed and not dependent on a minute to minute measurement of blood glucose. Although not small enough to be implantable these devices are small enough to be portable. The main problems with the open loop system are infection at the infusion site and potential hypoglycemic encephalopathy. (5,7)

Pump treatment has been associated with the correction of a variety of metabolic, functional and structural abnormalities that characterize poorly controlled diabetes, however clinical trials to date provide only conflicting evidence of their ability to prevent or reverse nephropathy and retinopathy.

WHY CONVENTIONAL TREATMENT OF DIABETES FAILS - ANATOMIC AND PHYSIOLOGIC CONSIDERATIONS IN THE SECRETION OF INSULIN

The ideal degree of control that must be obtained before it could be considered adequate would mean restoring glucose and insulin levels to those that would imitate the normal. Thus, when glucose levels are increased, insulin should respond appropriately to maintain the blood glucose level within a narrow range between 60 and 100 mgm per 100 ml in the fasting state and no higher than 150 mgm per 100 ml in the immediate postabsorptive state. In healthy fasting nondiabetic individuals insulin is secreted at a relatively low rate, probably less than 1 unit per hour to maintain peripheral insulin levels in the range of 10 to 14 units per ml. Prevention of hyperglycemia at meal times requires a rapid and properly timed increase in the insulin secretory rate that is proportional to the size of the meal. The insulin level may rise to a peak five to fifteen times the fasting concentration. To prevent postprandial hyperglycemia the burst of insulin secretion must anticipate the influx of glucose from the intestine rather than simply react to a rising plasma glucose level. (2,7)

The complexities involved in insulin secretion to maintain glucose homeostasis have just recently been recognized and are not as yet completely understood.

The islets of Langerhans constitute unique endocrine microorgans. (8) Approximately one million islets of Langerhans, 40 to 200 microns in diameter are dispersed within the acinar parenchyma of the pancreas. (49) The islets constitute 1.5 percent of the pancreas by weight. (50)

The individual islet is comprised of 75 percent beta (B) cells, 20 alpha (A) cells, 5 percent delta (D) cells and a small number of 'C' cells. (50) Each islet has a constant topographical arrangement of cells. (8,50) The cells are arranged in layers with the A cells outermost, D cells intermediate and B cells central. A cells are the source of glucagon, B cells the source of insulin and D cells produce somatostatin. Electron microscopic studies have documented the presence of bridges or 'gap junctions' connecting cell types within the islet implying physiological interactions between the cells. (50) In a functional sense each islet is a syncytium with individual cells coordinating the respective secretory activities of others. (8)

In health normoglycemia is a result of a coordinated interplay of many factors and several organs. For example, glucose itself is not normally the initiator of the insulin response early in the course of a meal. Insulin secretion begins before the first rise in arterial glucose. It is believed that several signals arise in the gastrointestinal tract including vagal neurotransmission and gastrointestinal polypeptides such as gastric inhibitory polypeptide, cholecystokinin, secretin and gastrin. (7) The importance of the hepatic portal circulation for insulin to maintain normal fuel metabolism has been demonstrated. (51) In a nondiabetic individual normoglycemia is largely maintained by a coordinated interplay of insulin and glucagon with insulin limiting the magnitude and duration of postprandial hyperglycemia and glucagon preventing hyperglycemia between carbohydrate containing meals. (7)

Studies have demonstrated that multiple daily insulin injections in combination with strict control of diet and exercise cannot achieve normal blood glucose levels in labile insulin dependent diabetics. (6) It is not surprising that attempts to mimic the complex control of fuel homeostatis by the peripheral administration of insulin titrated to peripheral blood glucose levels are unsuccessful. Exogenous insulin is unlikely to provide the degree of control necessary to prevent chronic complications in diabetes. (10)

THE IDEAL TREATMENT OF DIABETES - PANCREATIC TRANSPLANTATION

Total endocrine replacement therapy in the form of transplanting the whole organ, a segment of the pancreas, or the islets themselves (9) has been referred to as the ideal treatment of diabetes. (4,11)

Studies of experimental pancreatic transplantation over the last ten years have demonstrated the ability of grafts to prevent or reverse diabetic microangiopathy in small animals. (4,9,18,19,49,52-54) The experimental work of Mauer and coworkers (18,19) is frequently cited. They documented the effect of islet cell transplantation on established renal glomerular lesions. After islet cell transplantation to rats with diabetes of more than six months duration light microscopic lesions either failed to progress or there was an actual decrease in mesangial matrix material. Immunoglobulin and complement progressively disappeared as the metabolic abnormalities were corrected, while in untreated diabetic rats, the renal lesions continued to progress.

Recently Hoffman and associates (55) demonstrated that good control of diabetes in rodents with parenteral insulin was significantly less effective in the prevention of glomerular thickening when compared to islet transplantation. Pozza and coworkers (56) compared the metabolic effects of continuous subcutaneous insulin infusion, continuous intraperitoneal insulin infusion and pancreas transplantation in insulin dependent diabetic patients. Blood glucose control was unsatisfactory during continuous subcutaneous insulin infusion despite high levels of immunoreactive insulin. Continuous intraperitoneal insulin infusion and pancreas transplantation nearly normalized both blood glucose and immunoreactive insulin.

III PANCREATIC TRANSPLANTATION

The concept of transplanting the pancreas into human diabetic subjects is not new. The classic studies of Von Mering and Minkowski in 1889 demonstrated that removal of the canine pancreas resulted in hyperglycemia. (57) Since that time the possibility of transplantation of pancreatic tissue to ameliorate diabetes has excited experimental investigators. In 1892 Hedon described neovascularization of allografts of canine pancreatic fragments placed subcutaneously on a temporary vascular pedicle. Normoglycemia was unaffected by division of the pedicle but diabetes ensued following excision of the pancreatic graft. (49) The first recorded human pancreatic transplant was probably performed by Watson and Harsant on December 20, 1893. (58) They treated a fifteen year old boy by the subcutaneous implantation of three pieces of freshly slaughtered sheep pancreas, each piece "the size of a Brazil nut." The boy died after three days and histology of the transplant showed only "fibrous stroma". Sobolev's studies in 1902 are frequently quoted as the first to recommend transplantation in the treatment of diabetes. (59) Pioneering studies by Ivy and Farrel in 1926, Houssay in 1929 and Bolin in 1936 documented short-term function and histological survival of canine pancreatic allografts. (49) Following the report by Banting and Best in 1922 of reduction of blood sugar in a patient with diabetes mellitus given injections of a partially refined bovine pancreatic extract, most therapeutic approaches to the treatment of diabetes were centered on the optimal administration of exogenous insulin. (1) The relatively recent realization that exogenous insulin therapy does not prevent the long-term complications of the disease has led to renewed interest in alternative modes of therapy such as pancreatic transplantation.

There are two major efforts underway to develop the transplantation of insulin producing tissue for diabetic patients, immediately vascularized pancreas (whole organ or segmental) grafts and islet cell transplantation. Technical barriers have prevented widespread and safe clinical application of both techniques. The ultimate problem faced by each technique is allograft rejection.

PANCREAS TRANSPLANTATION (WHOLE ORGAN AND SEGMENTAL GRAFTS)

Lichtenstein and Barshak in 1957 were the first to describe immediately vascularized whole organ pancreatic transplantation in dogs. (49) Increasing familiarity with pancreatic and vascular surgery coupled with the early enthusiasm for human renal transplantation led to experimental studies of canine pancreas grafts involving a variety of implantation techniques and generalized immunosuppressive regimes.

Following the initial human pancreas allograft studies by Kelly, Lillehi and Merkel in 1966 whole organ allotransplants were performed with limited success. (49,60) Early clinical trials used pancreaticoduodenal transplantation in which exocrine drainage was maintained through the duodenum. (9,13,61) Technical problems frustrated these early efforts. The incidence of severe complications, often resulting in a transplant related death was in the order of fifty percent. The complications, a result of exocrine enzyme activity, included duodenal necrosis and perforation, anastomotic leaks and dehiscence, fistulas and sepsis. Vascular thrombosis and torsion were other common reasons for technical failure. (9,11)

Transplantation of the body and tail of the pancreas, the segmental pancreatic graft, was evaluated. This method proved to be technically easier than pancreaticoduodenal transplantation and avoided some of the technical problems related to the grafted duodenum. (9,13) Exocrine secretion was handled by anastomosis of the severed duct to the ureter (62) or to a defunctionalized loop of bowel.(63) The same problems of local necrosis and anastomic leak occurred.

Other methods of handling the exocrine secretion were subsequently devised. Ligation of the duct was evaluated experimentally (64) and clinically. (65) Peripancreatic accumulation of enzyme rich fluid complicated this approach as pressure in the obstructed duct was partially relieved by severed lymphatics. (61) Kyriakides and coworkers (66) demonstrated the technical feasibility of segmental pancreatic transplants with no attempt to control exocrine secretion, the duct allowed to drain freely into the peritoneal cavity. The open-duct technique has resulted in pancreatic ascites. (15) The most radical approach for avoiding exocrine leakage is injection of synthetic polymer into the ductal system as advocated by Dubernard. (67,68) This method prevents not only the escape of exocrine secretion but its formation. This method has been used in the majority of cases over the last few years and has the advantage of simplicity and a lower incidence of exocrine activity related complications. (69,70) A major problem with all the techniques that interrupt exocrine secretion is fibrosis and atrophy of the exocrine pancreas that ultimately leads to impairment of the endocrine function. (13,68,71)

The presence of multiple methods to handle the exocrine secretion of the pancreas indicates the magnitude of the problem. Recently enteric drainage, such as pancreatico-jejunostomy is being reconsidered on the basis that it is more physiological than duct occlusion and preserves the morphology and function of the pancreas. (11,13,68,72)

PANCREATIC ISLET CELL TRANSPLANTATION

The devastating complications of the early attempts to transplant the intact pancreas prompted the investigation of transplantation of isolated islets. From a theoretical point of view transplantation of the islets of Langerhans only would eliminate the problem of handling the exocrine secretions and the operative procedure would be less complicated.

The earliest attempts at islet cell transplantation were free grafts of pancreatic fragments. (73) Browning and Resnick in 1951 documented short-term functional and histological survival of islet containing allografts of minced embryonic murine pancreas. (74) In 1959,Brooks and Gifford on the basis of their studies of canine whole organ pancreatic allografts implanted minced pancreatic tissue from a stillborn infant into the quadriceps muscle of a diabetic mother. Transient reduction of exogenous insulin requirements was noted. (49) These initial attempts to transplant pancreatic fragments were considered unsuccessful and unsafe because the associated exocrine enzymes autodigested the transplanted tissue and injured the host. (73)

Experimental Studies In Rodents - Transplantation of Isolated Islets

Initial efforts in pancreatic islet cell transplantation were directed at isolating relatively pure preparations of islet tissue in rodent models. Microdissection techniques were being used to isolate islets for biochemical and physiological studies. (58) However these techniques were traumatic and produced only small numbers of islets. The need for islets for invitro studies prompted the work of Moskalewski. (75) He used the enzyme collagenase to digest chopped guinea pig pancreas. Although the enzyme destroyed many islets it did allow complete separation of islets from acini. The islets were viable and responded to invitro glucose stimulation by degranulation. Lacy and Kostianowsky (16) improved the technique by intraductal distention of the pancreas to mechanically disrupt the exocrine tissue prior to mechanical mincing and enzymatic digestion of the gland. They then separated the islets from the digested pancreas by centrifugation in sucrose gradients. These modifications resulted in the isolation of approximately 300 intact islets from a single rat pancreas. Lindall substituted layered Ficoll as the separating agent on the basis that it provided a better osmotic environment for the islets than the unpolymerized sucrose. (76) Subsequent modifications included dialyzing the Ficoll or adjusting the Ficoll to isotonicity with Hypaque to reduce injury to islets. (9) Some contamination with other tissue was inevitable but a relatively clean preparation of islets could be obtained by hand-picking the islets. Suspending the Ficoll separated islets in phenol red solution under a dissecting microscope with a reflected green light facilitated the process of hand-picking the islets. (77) The present methods used to isolate islets for experimental transplantation in rodents represent a composite of the above innovations.

Younoszai and associates were the first to report transplantation of isolated adult rodent islets. (9,58) They demonstrated temporary amelioration of chemically induced diabetes in rats. Ballinger and Lacy

(17) were the first to demonstrate a sustained and significant reduction of blood sugar following transplantation of isolated islets. The transplantation of approximately 400 to 600 islets to the peritoneal cavity or to the thigh muscle of inbred diabetic Lewis rats resulted in a significant reduction of hyperglycemia, glycosuria and restoration of weight gain. Excision of the transplanted islets from the thigh muscle resulted in a return to the fully diabetic state. Histological examination of the excised islets revealed intact beta cells with degranulation, which indicated to the authors the great demand for insulin on these cells. Reckard and Barker in 1973 demonstrated complete normalization of glucose in rats for as long as seven months following an intraperitoneal injection of 800 to 1200 isologous islets isolated from four to six pancreases. (23) Other investigators confirmed the findings that transplantation of a larger number of islets from multiple donors could completely normalize blood glucose in rodents. (9,24,78,79)

It was established that the latent period between transplantation and amelioration of diabetes is shortened and glucose tolerance improved as larger numbers of islets were injected. In general if more than 1000 islets were injected into the peritoneal cavity of a diabetic recipient normoglycemia occured within a day or two of transplantation. As only 200 to 400 islets could be isolated from one pancreas, multiple donors were required for a successful transplant. This was not a major barrier to experimental studies because of the existence of inbred strains of rodents. (9)

A major contribution to islet cell transplantation was made by Kemp and coworkers. (80) They demonstrated that the same number of islets, approximately 800, that only partially ameliorated the diabetic state when transplanted intraperitoneally could completely normalize plasma glucose levels and glucose tolerance tests when embolized to the liver by the portal vein. Matas and associates (81) as well demonstrated the superiority of the portal versus the systemic or intraperitoneal implantation site. They transplanted minimal quantities of islet tissue to isogenic adult rats with chemically induced diabetes. As the amount of transplanted tissue was decreased there were significantly more cures with the portal vein route. Either more islets survived the trauma of transplantation because a blood supply was immediately available or there was a physiologic advantage to this site because of the normal secretion of insulin into the portal system. Morphological studies demonstrated that implanted islets were widely distributed throughout the liver in peripheral interlobular portal venules and surrounded by vacuolated liver cells containing large stores of glycogen. The structurally normal endocrine cells derived a dual vascular supply from periportal and venous sources. Active innervation of intraportal grafts occurred in association with the process of vascularization. (82) The physiologic importance of the hepatic portal circulation for insulin activity was subsequently demonstrated. (51)

Isolated islets were transplanted to other sites with variable results. Intrasplenic injection proved to be nearly as effective as intraportal injection in rats. (83) Rats with intrasplenic autografts failed to exhibit a normal biphasic insulin response during an intravenous glucose tolerance test, unlike, intraportal autografts. As well as the intraportal, splenic and intraperitoneal sites, diabetes in rodents has been reversed by embolization of islets to the lung via a systemic vein, direct injection into the liver, implantation beneath the renal capsule and recently implanted in a surgically created peritoneal-omental pouch. (9,49,84) Diabetes has rarely been reversed after subcutaneous, intramuscular, intratesticular, intrapancreatic or intrasalivary gland implantation of islets. (9)

Shortly after Ballinger and Lacy reported their work in 1972, Leonard and Lazarow (85) showed that diabetes in rats could also be ameliorated by intra-peritoneal transplantation of multiple neonatal pancreases dispersed by collagenase digestion without specific islet isolation. Twenty to thirty-five neonatal donors were used for each transplant. The rationale for using neonatal pancreas was based on studies in their laboratories which demonstrated that neonatal pancreas possessed an extraordinarily low exocrine enzyme content and a relatively high percentage of islet tissue. The objective of the study was to develop an effective technique of pancreatic islet transplantation which circumvented prior islet isolation. Matas and coworkers (86) demonstrated, using the portal vein,that they could ameliorate diabetes with collagenase dispersed pancreatic fragments from as few as four neonatal donors. It took the plasma glucose from five to eight days to return to normal, the time interval inversely proportional to the tissue insulin content of the implanted tissue. The significance of the above work was demonstrating that diabetes in rodents could be reversed by transplantation of pancreatic fragments without specific islet isolation. The major problem was that for rapid amelioration of diabetes a very large number of neonatal donors were required. (9)

The use of foetal pancreas was investigated as well. (87-89) Even though the foetal beta cell mass was small the foetal pancreas had the same favorable ratio of high islet volume and low exocrine enzyme content as well as the ability to grow and differentiate post-transplantation. Most investigators transplanted foetal islets as intact free pancreas grafts placed beneath the renal capsule. Transplantation of intact foetal pancreas avoided the problem of enzymatic destruction of islets and thus required fewer donors, in general, an average of four to ameliorate diabetes. A latent period of several weeks between transplantation of the foetal pancreas and reversal of diabetes was necessary. This was felt to be a consequence of the need for growth and maturation of the endocrine component of the foetal pancreas. Mullen (87) demonstrated that one foetal pancreas could treat diabetes if it was placed under the kidney capsule of syngeneic normal rats and allowed to mature three weeks before enbloc transplantation of the kidney and intact pancreas to the recipient.

The results of the above series of investigations, reproduced in many other laboratories, established that complete and permanent reversal of diabetes in rodents was possible by the transplantation of islet tissue. Extensive metabolic studies demonstrated normalization of growth, plasma insulin, glucose tolerance and a variety of other metabolic factors. (9,49,78,79,90) Islet transplantation in rodents was demonstrated to halt and reverse the microangiopathy of diabetes. (18,19,41,52-54)

Experimental Studies in Large Animals - Transplantation of Pancreatic Fragments

The experimental success of islet transplantation in rodents prompted studies in larger animals, including primates, with the idea of applying islet cell transplantation to the treatment of diabetes in man. It soon became apparent that the fairly standardized rat isolation techniques would not work in the more compact and fibrous mammalian pancreas, in particular the human pancreas. (10,20,21,91-94) The yield of islets from one donor was insufficient to ameliorate the diabetic state. Unlike rodents, multiple donors could not be used as allogenic islets would be rejected before function of a technically successful graft could be determined.

Merkovitch and Campiche (20,91) partially solved the problem of islet yield. They reversed diabetes in 20 of 25 dogs with intrasplenic autografts of dispersed pancreatic tissue. The graft was prepared from the tail of the pancreas only. The gland was infiltrated with collagenase solution either insitu (15 dogs) or after excision (10 dogs) and then cut with a scalpel into two to four millimeter pieces. The particles were then incubated with collagenase for fifteen minutes in a shaking water bath at 37^{O}C. The prepared tissue was then implanted into the spleen of the same animal via a branch of the splenic vein. They chose the spleen as the site for transplantation because of its rich blood supply with venous outflow to the portal circulation.

Kretschmer and coworkers (21) demonstrated that normoglycemia occurred in 20 of 21 dogs receiving intrasplenic autografts of dispersed pancreas prepared from the entire gland. Graft preparation involved mincing the entire pancreas in a mechanical tissue chopper followed by collagenase digestion in a shaking water bath. Controls within the study established that collagenase digestion was a necessary part of the graft preparation for successful transplantation of pancreatic fragments.

Using the above techniques or modifications of the above techniques, intrasplenic autografts of pancreatic fragments prepared by enzymatic digestion and mechanical dispersion have been shown by other investigators to reverse the diabetic state in dogs. (22,95-101) However, there has been difficulty in demonstrating complete restoration of metabolic function. In the studies of Merkovich and Campiche (20) glucose tolerance tests in transplanted dogs were similar to controls but peripheral vein insulin levels were only half those of normal. In Kretschmer's series (21) the animals had fasting normoglycemia but the rate of decline of glucose during glucose tolerance tests (the K value) was only half that of the normal controls. Peripheral vein insulin levels were very low. In the studies of Warnock and coworkers (92) the K values at one and two months after transplant were only half those of the controls. Peripheral insulin levels were less than 25 percent of normal. More detailed studies of metabolic function of canine intrasplenic autografts have recently been reported. (99-101) Alderson and Farndon (100) demonstrated that impaired glucose tolerance at one month existed in association with abnormalities of lipid metabolism both in the fasting state and after intravenous glucose loading. An interesting observation is that islet cell grafts undergo functional improvement with time. (20,21,99,100) Rajotte and coworkers (101) established that the glucose receptor mechanisms, of transplanted islets, for release of insulin were intact three to five months after transplantation by demonstrating a biphasic release of insulin in venous effluent from the spleen. They concluded that the beta cell maintains its 'fine tuning' to prevent wide excursions in plasma glucose levels in response to a glucose challenge.

In the rodent embolization of isolated relatively pure islets into the liver was established as the most efficient site for transplantation. (80,81,83) Dispersed pancreatic fragments have been infused into the portal vein of pancreatectomized dogs. (97,98) It was noted that serum liver enzymes were transiently elevated and that the dogs tended to develop portal hypertension. Mehigan and associates (102) described disseminated intravascular coagulation following intraportal infusion of pancreatic fragments. The problems were felt to rise from the large fragment size and release of pancreatic enzymes into the vascular system. Miller and coworkers (103) investigated the cause of disseminated intravascular coagulation during dispersed pancreas autotransplantation. The most severe effects were obtained by injecting the collagenase used in the tissue preparation itself. Lorenz and associates (104) did not report elevation of liver enzymes or portal hypertension after intraportal injection of isolated islets (purified by Ficoll gradient separation). With respect to heterologous sites of transplantation successful engraftment of islets in a renal subcapsular position has been reported. (105) The superiority of the splenic bed as a transplantation site for pancreatic fragments has been emphasized by several investigators. (20,22,29,97)

The above work demonstrates that, by eliminating the steps of purification, sufficient islet tissue can be obtained from one donor for an effective transplant in the canine model. Long-term normoglycemia is achieved, however, there is not complete restoration of metabolic function. The problem of islet yield has only been partially resolved.

ALLOGRAFT REJECTION

A major problem common to both pancreas and pancreatic islet cell transplantation is the predictable occurrence of allograft rejection.

Pancreas (Whole Organ and Segmental) Grafts

Without immunosuppressive treatment the survival of canine pancreatic allografts is limited to a few days or weeks. Most investigators have administered various combinations of azothioprine, corticosteroids and antilymphocyte serum to recipient animals. The results are fairly uniform. In animals that do not succumb to technical complications the mean graft functional survival or recipient survival time in nonimmunosuppressed mongrel dogs has ranged from three to five weeks. (9)

Recently cyclosporine has prolonged functional survival of pancreas allografts in rats and dogs. (9,13) Clinically, a higher percentage of diabetic pancreas transplant recipients have functioning grafts than those immunosuppressed with azothioprine. (12) Cyclosporine has been associated with a two fold or greater pancreas allograft functional survival over that achieved with azothioprine. (70) As well as increasing graft survival cyclosporine has the advantage of less side-effects when compared to previous multi-drug immunosuppressive regimes. (12,13,106) Side effects of major concern with multi-drug immunosuppressive regimes include bone marrow suppression with azothioprine and impaired glucose tolerance with steroids for example. Cyclosporine treatment is not without complications. Of particular importance to diabetic patients receiving cyclosporine are its nephrotoxicity (106) and recent evidence that cyclosporine in high doses may cause a deterioration in glucose control by a dose dependent peripheral blockade of insulin. (107)

The major biological problem with pancreas transplantation at present is the need to use systemic immunosuppressive agents to prevent allograft rejection. (9,12,13,70) The majority of whole organ transplants have been accomplished in patients who were receiving immunosuppressive agents to maintain renal transplants. Uremia itself is immunosuppressive and in nonuremic diabetic patients receiving immediately vascularized pancreas allografts success with conventional immunosuppressive regimes has been nil. (12) Pancreas transplantation at present is limited to diabetic patients whose complications have progressed to a stage more serious than the potential side effects of antirejection therapy. (10,12,13)

Islet Cell Grafts

Because of the technical problem of islet yield in various islet cell transplantation models in large animals and man most information concerning the immunologic aspects of islet cell transplants stems from experimental experience with rodents.

With the advent of islet transplantation it was hoped that the islets were less immunogenic than the intact pancreas. (15,49) This optimism was based on prior observations that other endocrine tissue, such as parathyroid and ovary,evoked only a feeble immunologic response when transplanted. However, the earliest studies of transplantation of allogenic islets demonstrated that they were rejected very rapidly, surviving only a few days in the rat. (23,90,108)

Reckard and Barker (23) showed that islet allografts in rodents differing at major and minor histocompatibility loci were rapidly rejected within three to ten days. The studies of Nash and coworkers (109) suggested that islets were more susceptible to rejection than kidney, heart and skin grafts. Sutherland (9) reviewed nineteen separate studies of allografts in the rat model. Rat islets, transplanted across a major histocompatibility barrier survived as long as six days in only two of the studies. Minimizing histocompatibility differences at the major histocompatibility locus failed to extend rat islet allograft survival beyond eight days. In contrast vascularized pancreas grafts between major histocompatibility identical rats often survived longer than thirty days. (110) Studies in nonimmunosuppressed animals showed that the interval between transplantation and occurrence of hyperglycemia was longer with pancreas than islet cell grafts. (110,111)

Various types of nonspecific immunosuppression have been used in islet cell allograft experiments including azothioprine, adrenocortical steroids and cyclosporine. (9,15,49) All have proved relatively ineffective in prolonging survival of isolated islet allografts whereas the same or similar agents have almost uniformly proved to be more effective in prolonging survival of vascularized pancreas allografts. Specific immunosuppression in the form of antilymphocyte serum is more effective in extending allograft survival in whole organ transplantation than islet cell grafts. (15) Methods of inducing specific immunologic unresponsiveness such as injection of donor strain bone marrow cells to the recipient in the neonatal period extends vascularized pancreas but not islet cell allograft survival. (111)

Approaches uniquely applied to islet cell grafts and not whole organ transplants have been investigated. Islets have been transplanted to a number of 'immunoprivileged sites' such as the anterior chamber of the eye,the hamster cheek pouch, the testes and to a renal subcapsular position. (29,49,105) Except for a recent report of renal subcapsular islet transplantation in the canine model (105) the approach has not consistently led to prolonged allograft survival. Immunoisolation by encapsulation of islet allografts has been studied as another alternative to immunologic manipulation of the recipient. (9,49) Short-term survival of membrane protected islets has been documented. However technical difficulties with membrane permeability and islet number serve as significant obstacles to longterm graft function.

From the above discussion islets would appear to be particularly vulnerable to rejection. The explanation for this very brief survival of islet allografts has not been elucidated. One explanation is the cellular nature of the graft. (9 ,15) Other cellular grafts such as bone marrow are susceptible to damage by humoral immunity. (15) Zeigler, Reckard and Barker (90) demonstrated that recipients tolerant of islet allografts, rejected the islets, but not a skin graft within seven days after injection of recipient antidonor antibody. Another explanation is based on the fact that quantitatively less insulin secreting tissue is engrafted with islet cell transplants than whole organ grafts. (9,112) If the number of islets engrafted is just sufficient to ameliorate diabetes the destruction of only a few islets will result in a return to the hyperglycemia state. It has been demonstrated that by increasing the number of transplanted islets functional survival of islet allografts in rats can be prolonged. (112)

Recent experimental evidence suggests that despite the highly vulnerable nature of islet allografts they may in themselves be only weakly immunogenic and thus in a sense immunoprivileged. The evidence for this seemingly paradoxical situation is based primarily on experiments in which pretransplant treatment of islets with tissue culture techniques has been carried out to ensure transplantation of relatively pure endocrine tissue. (25,26,113-117)

The work on islets was stimulated by reports that pretransplant organ culture extended survival of other endocrine tissue allografts. Lafferty and associates (27) demonstrated that maintenance of mouse thyroid and parathyroid in 95 percent 02 for a period of three to four weeks before transplantation markedly prolonged allograft survival. Initial attempts to use the invitro techniques described by Lafferty on isolated adult rat islets were unsuccessful. (114) Exposure of the islets to 95 percent O_2 resulted in disintegration of the islets after four to five days of culture. The tissue culture technique was subsequently modified based on observations that lymphocytes lost their ability to stimulate allogenic lymphocytes invitro when maintained at 24^oC for four days. (117) Rat islets were cultured under similar conditions and it was found that they remained intact and functional after four days of low temperature culture. Lacy and coworkers (25) demonstrated that when histoincompatible rat islets were maintained at low temperature culture for seven days and transplanted into recipients receiving a single injection of antilymphocyte serum, 85 percent of the islet allografts survived for greater than 100 days. It was subsequently demonstrated that with further modifications of the tissue culture technique, tissue culture alone was capable of extending the survival of allografts without immunosuppression. (113,115)

The rationale for the improved results of islet allotransplantation following pretransplant culture is based on a theory, first proposed by Snell in 1957, that a small number of allogenic lymphocytes or 'passenger leukocytes' could stimulate a strong immune response. (26,28) It was possible that the host sensitization resulting from islet transplantation was actually caused by the 'passenger leukocytes' rather than the endocrine cells in the islets. This premise was substantiated by demonstrating the acute rejection of cultured islet cell grafts when recipients were injected with donor peritoneal exudate cells, a lymphoid cell population enriched with macrophages. (119) The donor lymphoid cells initiated host recognition of the foreign or transplant antigens of the graft which served as targets for immune rejection. Thus even though cultured islets still appeared to express foreign transplant antigens, an immune rejection response was not initiated without donor lymphocytes.

Further investigation established that the islets might in fact be deficient in certain histocompatibility antigens. Two functional groups of antigens coded by the major histocompatibility locus have been examined by various techniques. Class I antigens (H-2K and H-2D in rodents, HLA-A, HLA-B and HLA-C in humans) are believed to be present on most nucleated cells and are target antigens in transplantation rejection. In contrast Class II antigens (Ia in rodents; HLA-DR in humans) are believed to control cell-cell interactions and the proliferative response of the recipients lymphocytes that ultimately destroy the graft. (117) Parr (120) using an immunoferritin labeling technique was unable to demonstrate any H-2 antigens on the surface of beta cells of dissociated mouse islets while high concentrations of H-2 antigen were found on acinar, ductal and capillary endothelium. Faustman and associates studying rodent islet cells with cytotoxicity and absorption assays found that H-2D and H-DK antigens were present on islet cells, however no Ia antigens could be demonstrated. Subsequent studies of mouse, rat and human islets have confirmed that Class I antigens are present but Class II antigens are lacking on islets of Langerhans. (117) Hart and coworkers (121) used monoclonal antibodies to identify Class I and Class II antigens on rat pancreas and pancreatic islet cells. They noted that interstitial dendritic cells, or fixed tissue macrophages stained intensely for Class II antigens. They postulated that the immunogenic dendritic cell represented the immunogenic passenger leukocyte.

At the present time it is felt that islet cell grafts, depleted of dendritic cell function (passenger leukocytes) by a period of tissue culture can be allografted presenting only Class I antigens to the recipient. Having been depleted of Class II antigens necessary to provoke an immune response the transplanted islets would not be rejected even by nonimmunosuppressed recipients. However they would be rejected if the recipient is confronted with donor Class II antigens at some point before or after the islet transplant. (10,15,26) The implication of the above work is that islet cell grafts could be safely used to treat diabetic patients shortly after the onset of their disease and prevent the development of microvascular complications.

AUTOIMMUNITY AND INSULIN DEPENDENT DIABETES

Recent evidence suggests that the pathogenesis of insulin dependent or Type I diabetes has an important autoimmune component. (15,33,122) Another potential biological barrier to successful pancreatic transplantation is recurrence of the original disease process in the transplanted tissue. In kidney allografts destruction of the transplant by recurrent glomerulonephritis occurs with uncertain frequency. (15) An animal model of spontaneous diabetes, the 'BB' Wistar rat allows examination of the recurrence question. A variable number of rats in this inbred strain develop a diabetic syndrome of abrupt onset characterized by severe insulopenia and mononuclear infiltration of the islets (insulitis). Naji and coworkers (33,122), using this model, have examined the vulnerability of islet cell grafts to autoimmune destruction. In their studies, islet cell grafts compatible at the major histocompatibility locus suffered autoimmune destruction when rejection was excluded by experimental design. Weber and associates (133) demonstrated that autologous islets survived when transplanted into rats with chemically induced insulitis. Clinical experience to date suggests that recurrent disease is not the obligatory outcome of islet cell grafts in humans. (15) It is hoped that the timing of human transplantation after the onset of the disease and the availability of immunosuppressive agents will diminish the possibility of destruction of grafted islets by autoimmunity.

A COMPARISON OF PANCREAS AND PANCREATIC ISLET CELL TRANSPLANTATION

Currently islet cell transplantation is only successful in experimental animals. (10,11,12,70) In animal models islet cell transplantation is more successful than whole organ or segmental grafts. A recent study (124) in rats demonstrated that isografts of intraportal isolated islets were metabolically more effective than segmental pancreas isografts of a similar beta cell mass. As well, the metabolic function of pancreas grafts, deteriorates with time. (13,72) Successful islet autografts improve metabolically with time. (21,99,100)

At the present time, clinically, only immediately vascularized pancreas grafts are able to obviate the need for exogenous insulin in diabetic patients. (11,12,69,70) Data from the transplant registries (American College of Surgeons - National Institutes of Health Organ

Transplant Registry; New Pancreas Transplant Registry) demonstrate that the success rate of immediately vascularized pancreas allografts is improving. (11,69,70) Between December 17, 1966 and June 30, 1984, 485 immediately vascularized pancreas transplants have been performed on diabetic patients at 52 institutions. Four hundred and twenty-five of these were performed since 1977. Of these, 297 patients are listed as alive with functioning grafts and 54 functioning for more than one year. The one-year actuarial patient and graft survival rates since July 1, 1977 are 75 and 29 percent respectively. Graft and patient survival are significantly higher since 1977 than before that time, and the graft survival rates for transplants performed in 1983 and 1984 are significantly higher than for cases performed between 1977 and 1982. As of August 1, 1984 the longest duration of function with an immediately vascularized pancreas graft has been slightly more than six years. This patient is still alive with a functioning graft. In contrast no recipient of a free pancreatic islet allograft is insulin independent. The total number of islet cell grafts performed, as of August 1, 1984 was 166. Only 7 new cases have been reported since July 1, 1983, all free grafts of cultured foetal pancreatic tissue performed at one institution. In the best studied series of islet cell grafts, 18 patients at the University of Minnesota insulin independence for more than four days was not achieved.

Despite the lack of clinical success islet cell transplantation has several important advantages over pancreas transplantation that justify extensive evaluation of the former approach. These advantages include: potential for reduction of graft immunogenecity in vitro prior transplantation; the ability to cryopreserve and bank tissue for future use; and the relative simplicity of the procedure, particularly in terms of patient safety.

The most significant and important of the above advantages is the ability to reduce graft immunogenecity in vitro. The work of Lacy (25,113, 114) and others (27,115-117) in the field of immunoalteration has demonstrated that islet cell allograft survival can be prolonged without immunosuppression. The necessity for systemic immunosuppression to prevent rejection of immediately vascularized whole organ grafts limits this approach to patients with complications that would otherwise progress to a stage more serious than the potential side effects of antirejection therapy. (10,12,13) Potentially, islet cell grafts, with a reduced or no requirement for systemic immunosuppression, could be transplanted to a diabetic patient shortly after the onset of the disease and prevent the devastating microvascular complications, the ultimate goal of pancreatic transplantation. (10,15)

For cadaveric organ transplantation to be successful on a large scale tissue preservation is an essential consideration. For whole organ preservation the techniques available are cold storage in a physiologic salt solution or hypothermic pulsatile perfusion with a plasma-like solution. The later technique is difficult because the pancreas is a low flow organ and commercially available machines are designed for high flow rates which result in considerable oedema of the gland. (9) In animal models cold storage of pancreas grafts for up to forty-eight hours is possible. Clinically, function of grafts stored beyond twenty-four hours cannot be achieved. (12,125) Three methods of islet preservation have been evaluated experimentally: tissue culture; cold storage; and cryopreservation. The first two methods provide only short-term alternatives for storage comparable to what is available for whole organ grafts. With the technique of cryopreservation a variety of cells and tissue fragments have been frozen and stored for months or years without evidence of loss of viability. (9) Cryopreserved (frozen-thawed) rodent and canine islets have been demonstrated to respond to glucose invitro and restore normoglycemia in diabetic recipients. (126,127) Protocols for freezing and thawing human islets are presently being evaluated. (128)

Although the complications of immediately vascularized pancreas grafts are decreasing it cannot be considered a safe procedure. The incidence of severe complications, often resulting in a transplant related death is still in the order of 20 percent. (11,69,70) Complications have been reported following clinical attempts of intraportal grafts of pancreatic fragments.(102,129) The complications have been attributed to the contaminating exocrine tissue. A pure suspension of islets combined with the relative simplicity of the transplant procedure itself should make islet cell transplantation safer for the recipient than immediately vascularized pancreas grafts.

IV BARRIERS TO CLINICAL ISLET TRANSPLANTATION

There are two major barriers to widespread and safe clinical trials of islet cell transplantation. The first is obtaining a high yield of islets from the fibrous mammalian pancreas. The second and ultimate problem is allograft rejection. For immunologic reasons as well as the safety of the transplant procedure itself a pure preparation of islets is desirable. The solution to the above problems is interrelated. The major goal of most experimental work at present is directed at finding a practical and efficient means of separating the endocrine component of the pancreas from contaminating exocrine cells and immunogenic cells including the vascular and ductal endothelium.

INCREASING ISLET YIELD

The work of Merkovitch and Campiche (20) demonstrated that it was possible to ameliorate diabetes in the canine model with islet containing tissue from one donor. This was accomplished by transplanting pancreatic fragments prepared by collagenase digestion and mechanical dissosciation, eliminating the steps used for graft purification in the rodent model. The problem of islet yield was only partially solved however. In the above study and in subsequent experiments (20,21,92,99, 100) it was evident that metabolic function in diabetic recipients of pancreatic fragments was only partially restored. As a consequence different aspects of the islet isolation process have been examined and modified in an attempt to improve islet yield.

The shortcomings of the methods used to prepare dispersed pancreatic fragments were recognized coincident with the evolution of the technique.

Ductal distention and disruption exposed interlobular plains to collagenase which digested exocrine and endocrine components indiscriminantly. (92) Mincing the pancreas resulted in a 50 percent loss of tissue insulin. (20) Incubation of the chopped pancreatic fragments in collagenase destroyed more than 70 percent of the islets as well as the acinar tissue. (86) The yield of islets obtained by mechanical dispersion and enzymatic digestion of the pancreas was less than 10 percent of the total islet cell mass of the original gland. (95)

The collagenase digestion technique was critically examined in an attempt to optimize the digestion process and improve islet yield. Kretschmer and associates (21) established that twenty minutes of collagenase digestion appeared to provide the optimal balance between the need for tissue dispersal and the preservation of islet cell mass in preparing pancreatic fragments. Mehigan and coworkers (96) concluded that particle size and collagenase lot were critical variables while the methods of mincing the gland and the concentration of collagenase did not appear to be important.

Scharp and associates (92,93) were the first to emphasize the primary problem with the collagenase digestion technique. This problem was controlling the collagenase digestion in such a fashion that a maximal number of islets are released. Islets isolated before this critical time would still remain entrapped in the undigested pancreatic tissue and isolation after this critical endpoint resulted in enzymatic damage to the islets. Identification of this critical endpoint was difficult and depended on the activity of each batch of collagenase, the age and type of donor animal and the experience of the investigator. With the above considerations in mind,Scharp (93),described a new digestion-filtration technique for islet isolation in larger animals that eliminated the need to identify a single endpoint. Pancreatic fragments were incubated with collagenase in a mesh basket with pores 280 microns in size, allowing islets separated from acinar tissue early in the digestion period to escape from over digestion into an outer chamber. Large particles of undigested pancreas were retained. The technique was shown to increase the yield from a single rat pancreas from 150 to 450 islets making possible successful treatment of diabetic recipients with islets obtained from only two donors. Using this method they improved the diabetic state in five Rhesus monkeys made diabetic by partial pancreatectomy and streptozotocinin. A single donor was used for each transplant.

Another modification was described by Horaguchi and Merrel (95) They introduced a predigestion step prior to islet isolation. This was accomplished by retrograde perfusion of the ductal system with collagenase. The partially digested gland was then chopped with scissors, mechanically dissociated in a shaking water bath and finally filtered through a stainless steel screen of 400 micron pore size. Residual tissue was further digested with trypsin. With this method, in the canine model, a 57 percent recovery of beta cell mass was suggested by insulin recovery. Compared to the intact gland a sixfold purification of beta cell content was noted. They achieved normoglycemia in three of five dogs transplanted to the liver and in two dogs which were transplanted to the spleen. The importance of this predigestion step prior islet isolation has been confirmed by others. (22,29)

Scharp and associates subsequently modified their initial digestion-filtration technique by incorporating a predigestion step and automating the filtration process. The result of these modifications was the autoisolator. (10,29,120) The device has five stainless steel screens for the pancreatic fragments. It is connected to a reservoir of enzymes which is pumped on to the screens and across the tissue fragments. Automatic mixing of the tissue assists the islets in passing through the screens and out of the autoisolator. In the canine model, grafts prepared by this method are successful in over 90 percent of single-donor, single-recipient islet autotransplants, however, glucose tolerance tests are not completely normal. (10,130)

A recent and novel development by Lacy and associates (131), using the universal fastener, Velcro, has produced large quantities of relatively pure islets. The tiny hooks on the material hold onto the fibrous portion of the pancreas permitting the collagenase to release intact islets. This method was initially evaluated on isolation of islets from the beef pancreas. Subsequent studies revealed that this method was more efficient than the autoisolater. (10) Presently efforts are underway to automate this process. (29)

Other approaches to optimizing the digestion process have been evaluated. Downing and coworkers (92,132) evaluated distention of the dog pancreas through the venous system of the gland as opposed to the ductal system. They reported a four-fold increase in islet yield. Their explanation for this improvement was that the intralobular disruption provided by the venous distention resulted in more efficient collagenase digestion. Other enzymes such as trypsin (95,133,134) and dispase (133) have been evaluated.

On the basis that collagenase destroys islets and contaminates the graft preparation investigators have evaluated the feasibility of preparing islet rich pancreatic fragments by eliminating enzymatic digestion altogether. Hinshaw and associates (135) reported isolation of five hundred thousand to two million islets from a single human pancreas by hand pressing undigested pancreatic fragments, three to five millimeters in diameter, through a stainless steel screen, 200 to 280 microns pore size. Hasiguchi and associates (136) incubated intact porcine pancreases for two to four hours at 37°C prior islet isolation. Islet cells retained their morphological and functional integrity whereas acinar cells became necrotic. Because of the acinar destruction the pancreas became soft and amenable to mechanical separation by repeated passages of pancreatic fragments across a stainless steel mesh incorporated in a syringe. Quantitative data on islet yield was not reported in this study.

The mechanical dispersion aspect of graft preparation has not received much attention. Gray and coworkers (137) recently described a modification of the digestion-filtration process that they have applied to isolation of islets from the human pancreas. They emphasized that their method did not involve mechanical chopping of the gland. The gland was digested by intraductal injection of collagenase and cut into fragments with scissors. These fragments were then dispersed by teasing apart the fibrous perilobular tissue releasing numerous fine fragments into the surrounding cold Hank's solution. These fine fragments were further dispersed by passing them through fourteen and fifteen guage needles. The islets were then retrieved by a series of filtrations through nylon mesh screens of various pore sizes and the use of Ficoll to separate the smallest islets. Using this method they isolated an average of 1001 islets per gram of human pancreas.

Another approach to increasing islet yield has been to manipulate the exocrine content of the pancreas prior to graft preparation. It has been demonstrated in rodents that by pretreating donors with agents that selectively injure exocrine cells (138) or degranulate exocrine cells (139) iselt yield can be increased. For example Payne and associates (138) pretreated donor rats with DL-ethionine, an agent that selectively injures exocrine cells by interfering with methionine metabolism. Dispersed pancreatic tissue from one donor ameliorated experimental diabetes in up to four recipients and allowed tissue to be preserved by culture for up to forty-eight hours without specific islet isolation. Vrobova (139) and associates pretreated donor rats, one to one and one-half hours prior islet isolation, with pilocarpine to degranulate exocrine cells. Islet yield was doubled. As DL-ethionine is a carcinogen and pretreatment of cadaveric donors is not a practical consideration, Sharp and associates (29) added pilocarpine, secretin, and pancreazymin to the ductal perfusate of the canine pancreas prior islet isolation. They reported an increase in islet yield.

In general the methods being evaluated to isolate islets in large animals and man provide a sufficient quantity of islets to achieve normoglycemia in dogs, but the islets are so few in number or so contaminated with exocrine cells that they are risky for clinical trials. (10)

INCREASING ISLET TISSUE PURITY

A pure graft preparation is desirable for immunologic reasons and the safety of the transplant procedure. The purification process used successfully in rodents, density gradient sedimentation using Ficoll followed by handpicking islets, is less efficient for human and dog tissue. (9,29) Lorenz and coworkers (104) appear to be the only investigators claiming any success with Ficoll separated islets in the canine model. Other methods of purifying dispersed pancreatic fragments have been evaluated.

Prior to the emphasis on tissue culture as a means of immunoalteration, this technique was evaluated as a means to separate or isolate islets from contaminating exocrine cells. The work was prompted by the studies of Lazarow and associates (140) who demonstrated that culture of human foetal pancreas resulted in disappearance of acinar cells and enzyme production while islet cell mass was preserved. Investigators confirmed similar findings with minced adult rat (141), canine (141,142) and human (141-145) pancreas. The acinar cells were rapidly autodigested due to the action of instrinsic digestive enzymes, while the islet cells, lacking these enzymes were relatively spared. The result was selective purification of the islet tissue.

Matas and coworkers (141,142) reported their experience with tissue culture of canine pancreatic fragments. They supplemented the culture media with pilocarpine, cobalt chloride and aprotonin. They measured the insulin and amylase content of the cultured tissue on the assumption that the insulin per gram of tissue was proportional to islet cell mass and that the ratio of insulin to amylase was an index of graft purity. The insulin to amylase ratio did not change during the first four to to eight hours of tissue culture because of a decline in both tissue insulin and amylase levels, but with continued depletion of tissue amylase the insulin to amylase ratio increased more than six-fold over the whole pancreas. The insulin content however had decreased by 50 percent compared to the insulin content of the pancreas after twenty-four hours of tissue culture. Fifteen dogs rendered diabetic (14 by partial pancreatectomy and streptozotocin;1 by total pancreatectomy) recieved autotransplants of pancreatic fragments cultured for twenty-four hours. Approximately one-third of the graft was injected intraportally and the remainder was dispersed intraperitoneally in each dog. Seven dogs were rendered normoglycemic. Six dogs survived to thirty days and were normoglycemic at that time. The dog rendered diabetic by total pancreatectomy was normoglycemic for two weeks.

Another approach to purification of islet cell grafts is the production of pseudo-islets. This technique is presently being evaluated by Scharp and associates.(19,29,147, 148) Formation of pseudo-islets involves initial digestion of the gland with collagenase and trypsin to a single cell preparation. The isolated islet cells are then purified, in the initial studies with discontinuous Ficoll gradients, and subsequently with the Beckman elutriator. The elutriator works on the principle that counter-current centrifugation separates particles of different size. (149) Gyrorotational culture is then used to reaggregate the isolated and purified islet cells into pseudo-islets. The principle of gyrorotational culture was initially described by Moscona. (148) He emphasized that when cells were placed in gyrorotational tissue culture, there was a process of selective aggregation of like cells with each other followed by a phenomenon called sorting out of cell types. The sorting out occurs via cellular migrations, recognition and adhesion. Using the above procedure a very pure graft preparation can be obtained. Autotransplants of pseudoislets in diabetic dogs restores normoglycemia in 25 percent of cases. (29)

Several other means of islet tissue purification are currently being investigated. Scharp and associates have been investigating a new flow-through electrophoreises device developed by the McDonnel Douglas Corporation. Preliminary studies have demonstrated the feasibility of partially separating individual islet cell types from each other. The fluorescent activated cell sorter has been shown to partially purify islet cells. Another approach is to use monoclonal antibodies directed at islet tissue. The antibodies are bound to plastic dishes or columns. The cell preparation to be purified is either passed through the columns or incubated in the prepared dishes. The antibody complexes with the appropriate cell type and retains them. (29)

The problem with all the above techniques is that islet tissue is lost during the purification process. As a consequence it is not possible to consistently restore metabolic function with purified grafts in the canine model and the yield of islets from the human pancreas has been too low to permit clinical trials.

INCREASING ALLOGRAFT SURVIVAL

The majority of recent investigations addressing the problem of allograft rejection have been in the field of immunoalteration, extensions of the initial work of Lafferty and Lacy. The emphasis has been on prolonging allograft survival without immunosuppression.

Approaches to invitro immunoalteration of islet cell grafts essentially involve finding selective methods of eliminating passenger leukocytes or dendritic cells prior to transplantation. The initial study reported by Lacy and coworkers (25) demonstrated that donor rat islets cultured at 24^{o}C for seven days in conjunction with a single injection of antilymphocyte serum transplanted into allogenic diabetic recipients would result in a 100 percent survival rate at 100 days. Since this report four additional means of immunoalteration have been described. Bowen and associates (115) showed that aggregates of 50 or more mouse islets or mega-islets, unlike isolated islets, would withstand exposure to 95 percent O_2 for a period of seven days. This treatment prevented rejection of islet aggregates over a three month period of observation when transplanted across a major histocompatibility barrier in mice. Lacy and coworkers (113) subsequently demonstrated that rat mega-islets would withstand exposure to 95 percent O_2 for a period of seven days in tissue culture. Xenografts of these cultured rat mega-islets beneath the renal capsule of diabetic mice resulted in a 40 to 50 percent survival rate at 70 to 90 days after transplantation. Another method, described by Faustman and coworkers (150) uses monoclonal antibody directed against Ia antigens located on the donor immune cells. Incubation of donor mouse islets with Ia antibody followed by complement produced 100 percent survival at 200 days after transplanting the mouse islets across a major histocompatibility barrier. Lacy (26) reports the studies of his group examining the effect of treatment of donor islets with antidendritic cell antibody. Pretreatment of donor mouse islets with antidendritic cell antibody and complement prevented rejection of mouse islet allografts in 85 percent of recipients at 70 days after transplantation. A fourth method was recently described by Hardy and coworkers. (151,152) These investigators showed that ultraviolet irradiation of islet cell grafts combined with twenty-four hours of tissue culture and perioperative cyclosporine could indefinitely prolong islet allograft survival in rats.

Immunoalteration studies are currently being evaluated in large animal models. Mullen and associates (153) reported their experience with collagenase digested mini-pig pancreas cultured for seven days at either 24°C or 37°C. As detected by monoclonal antibodies, Ia bearing cells were significantly decreased but still present after the period of tissue culture. They were unable to prolong allograft survival transplanting these islets across major histocompatibility barriers. They concluded that the treatment necessary to remove dendritic cell function from islet cell grafts will not be practical to the treatment of diabetes because of the great loss of islets. Lacy's group has not attempted to demonstrate prolongation of functional allograft survival in the canine model using the theories of immunoalteration. They feel that these investigations cannot be undertaken until the problem of islet yield is solved in large animals and man. They have however, documented a 75 percent histological survival rate of allografts of seven day cultured canine islets at two to three weeks after transplantation as compared to a 25 percent survival rate in untreated controls. They conclude that the passenger leukocyte concept does apply to dogs and that one of the methods of immunoalteration successful in rodents should also prevent rejection of canine allografts. (26)

Because of the problem of islet yield there has been a renewal of interest in other methods to prolong allograft survival. Again the emphasis is on minimizing systemic immunosuppression.

Two methods of immune enhancement or treating the recipients to induce a tolerant state have recently been described. The first regime induces unresponsiveness in adult mice by immunizing them with donor blood treated with antiserum to Ia antigens prior to transplantation of islets. This regime alone has produced greater than one hundred day survival of islet allografts transplanted across a major histocompatibility barrier. Preimmunization with untreated donor blood caused an accelerated rejection. (154) The second method uses ultraviolet light to treat donor rat blood prior to using it for preimmunization of the recipients. This treatment alone has produced greater than 160 day survival of rat islets transplanted across a major histocompatibility barrier. (155)

There has been renewed interest in transplantation to immunoprivileged sites, particularly the renal subcapsular area. Prolonged xenograft survival of rat islets has been demonstrated in a renal subcapsular position. (116,156,157) There are preliminary findings that the renal subcapsular space provides an immunoprivileged site for islet cell allografts in the dog. (158)

V RADIATION AND THE PANCREAS

A review of radiobiology and the effects of radiation on the pancreas suggests that radiation may be of value in overcoming the barriers to clinical islet cell transplantation. The studies reviewed suggest that radiation in moderately high doses can selectively injure exocrine cells and leave endocrine cells viable.

RADIATION BIOLOGY

Radiation biology is concerned with the fundamental process by which radiation interacts with living matter.

The spectrum of radiation includes two distinct forms of energy production. (159) The first, electromagnetic radiation, comprises a wide range of wave propagated energy and of particular interest are those forms used clinically, the roentgen (X) and gamma rays. X-rays and gamma rays are quite similar in their interactions but differ in their origin. The former are machine generated while gamma rays are emitted by the spontaneous decay of radioactive nucleotides such as naturally occurring radium and uranium and the man-made radioisotopes, such as cobalt. Both X-rays and gamma rays are extremely energetic and very penetrating having characteristics of both waves and particles. The second, particulate radiation, consists of fast moving particles (alpha and beta), neutrons and protons. These particles of definite mass and charge are given off by both natural and artificially produced radioactive elements, the process of fusion as in atomic reactors, particle accelerators, and may also be derived from the interaction of electromagnetic radiation with other matter.

The familiar unit roentgen or 'r', is used to measure x-rays and gamma rays. These units are measured in air directly from their source by means of an ionization chamber. It is defined as the quantity of radiation required to induce an emission equivalent to one electrostatic unit of charge in one cubic centimeter of air. This unit cannot be applied to neutrons and the more important aspect of radiant energy is the quantity absorbed by tissue. The term 'rad' has therefore been adapted for all kinds of irradiation. It represents the absorption of one hundred ergs of energy per gram of irradiated material. To equate the biological endpoint of the different types of radiation the term relative biological effectiveness (RBE) has been introduced. It relates the biological effect of a given dose of radiation from one source to the biological effect of the same dose from a standard source such as a 250 kv X-ray machine.

Initial mechanisms of radiation injury - biophysical events

Radiation deposits its energy in matter by both ionization and excitation. Ionization is the displacement of an electron from an atom or molecule. Of the two, ionization causes the most significant biological alterations. Radiation deposits energy randomly in discrete events manifested as clusters of ionizations. While the total energy deposited by biologically relevent doses of radiation is very small, each energy deposition can be ten to twenty times larger than chemical bond energies, more than sufficient to induce biochemical alterations. (159,160,161)

Although it is known that radiation causes ionization of matter the precise mechanism by which radiant energy exerts its biological effect on the cell is unknown. Radiation induced ionizations lead to a sequence of chemical changes in their vicinity that may result in damage to biologically important molecules of the cell. Such molecules when present in a solvent such as water can be affected by radiation in one or both of two ways. (160) They can be affected directly if the initial radiation interaction occurs in the molecule itself, or indirectly,by chemically reactive products produced by radiant energy absorption in nearby water molecules. Since about eighty percent of the mammalian cell is water it is not surprising the indirect mechanisms predominate in producing biologically important damage in the cell. (160, 162-164)

Ionization of a water molecule leads to the production of several major reactive species: the hydroxyl (OH-) and hydrogen ($H^{\cdot}$) free radicals and the aqueous or hydrated electron ($e^{-}aq$). Emerging evidence indicates that the $OH^{\cdot}$ radical is the major damaging species and hydroxyl radical damage to biologically important molecules may be primarily the result of hydrogen abstraction ($RH + OH^{\cdot} \rightarrow R^{\cdot} + H_2O$). The resulting organic radical product ($R^{\cdot}$) may then undergo further reactions that tend to fix this damage to an irreversible chemical alteration. (160,161)

Idealized models have been developed from studies on simple homogenous populations of cells in which radiation effect in any one individual is independent of events in other members. As far back as 1924 Crowther proposed a cellular model assumed to contain a radiation sensitive volume or target. (164) Target theory proposes that radiant energy acts by direct hits or direct action on the target molecules within the cell. A single hit ionizes and inactivates a single vital component or substance damaging or killing a cell. With only one critical target per cell, target theory predicts an exponential cell survival as a function of radiation dose. However such an exponential form is not usually found for mammalian cell inactivation. An initial shoulder in the survival curves suggests that multiple radiation events are necessary to inactivate the cell, either one sensitive site to be hit twice or more, or for one hit to occur in two or more sensitive sites in the cell. These possibilities are referred to as the multihit or multitarget theory. Analysis of data from most cell survival curves support the multitarget theory. (160,164)

The transfer of energy to a target atom or molecule from the source of radiation occurs within microfractions of a second, yet the biological effect may not be apparent for months or even decades. Radiation effect therefore has a latent period. It is assumed that sequential reactions occur which ultimately exert a detectable functional or morphological effect. The indirect theory as a mechanism, and the multitarget theory as a model for radiation injury relate better to this latent effect. (159)

The biological target - damage to living cells

The tissues of the adult human body contain about five trillion cells. (163) These cells are the basic biological units and represent the biological target of ionizing radiation.

All mammalian cells have certain common features of organization such as a nucleus (except mature erythrocytes) which is separated from the cytoplasm by a nuclear membrane except at the time of cell division. Other common features include the mitochondria for production of energy, ribosomes for synthesis of proteins, the Golgi apparatus active in secretory cells, the endoplasmic reticulum where secretory proteins are synthesized and lysosomes representing the digestive system of the cell. Surrounding all this is a lipoprotein membrane whose integrity is essential to the function of the cell. (161,162)

Proteins are intimately involved with all cellular functions, including the structural elements of the cell, enzymes which catalyze essential chemical reactions, and many of the hormones which regulate metabolic processes. Simple proteins are chains of amino acids while conjugated proteins contain an organic chemical moiety in addition to the amino acids. For example nucleoproteins contain nucleic acids plus amino acid sidechains and glycoproteins contain carbohydrates in addition to amino acids. Loss of function of a protein from radiation injury is not usually due to breaking peptide bonds or otherwise disrupting the primary skeletal structure of the peptide chain. It may result from a change in a critical side chain or from a break in the hydrogen or disulfide bonds which maintain the secondary and tertiary structure. Such a break can lead to partial unfolding of the tightly coiled peptide chains which in turn can result in a disorganization of the internal structure, a distortion of necessary spatial relationship of side chain groups, or an exposure of amino acid groups resulting in a change in chemical activity. The hydrogen bonds of the secondary and tertiary structure are weak bonds. A number of them will be temporarily broken in the vicinity of an ionization. One primary ionization can alter the structure of a molecule and lead to an extensive change in overall chemical reactivity, (163,164)

Cumulative evidence suggests that deoxyribonucleic acid is the most vulnerable target to radiation. More specifically the linkage and bonds within the deoxyribonucleic acid molecule are vulnerable producing a variety of lesions including single strand breaks, double strand breaks, and various base alterations. The result of this is inhibition of deoxyribonucleic acid synthesis and interference with and delay of the mitotic process. In the clinical range of acute radiation dosage this represents the single most important insult to the cell. (159,160, 169) As well, because of chromosomal injury radiation has the potential of inducing neoplasia. (159)

The macromolecules within membranes, enzymes and other constituents are also damaged by radiation. (159,163,165) With large doses of radiation there is severe damage to the cell membrane. Alterations in cellular permeability after radiation are well known. Boeg and Alexander believe that many of the severe effects of radiation on cells are caused by damage to the macromolecules constituting both the intracellular and intercellular membranes. (165) Again, after high doses, a reduction in enzyme synthesis may be evident as a depression in protein synthesis. This may occur without noticeable cellular changes. (163)

The morphological counterparts of the injuries described above involve both the cytoplasm and the nucleus, but the latter, as expected, is more radiovulnerable. During the initial response to radiant energy there is cellular swelling, cytoplasmic vacuolization and alterations in plasma membranes. Mitochondria enlarge and assume distorted shapes. These mitochondrial changes may be secondary to other metabolic dislocations since many observations suggest that mitochondria themselves are relatively radioresistant. The endoplasmic reticulum is usually affected. Lysosomes appear to be more resistant and are often increased in number. Nuclear changes are marked and include nuclear swelling, vacuolation, focal disappearance of the nuclear membrane and in severely affected cells nuclear pyknosis or lysis. The cell itself often assumes bizarre sizes or shapes sometimes with formation of giant cells containing a bizarre pleomorphic nucleus or more than one nuclei. (159)

Radiation induced cell lethality

There are two modes of radiation induced cell lethality: mitotic death and interphase death. Cells receiving a lethal but moderate radiation dose will ultimately fail to pass through mitosis, possibly after one or more relatively normal mitosis. Such cells are said to undergo a mitotic death. Mitotic death is primarily responsible for the acute effects of radiation on whole animals. Its consequences predominate at the radiation dose levels used in radiotherapy of tumors since tumor cells need only to be reproductively inactivated to prevent further tumor growth and metastases. The mitotic death endpoint is only relevent to cells that have a proliferative capacity. Doses as small as twenty-five rads may cause necrosis of frequently dividing cells. In a few sensitive cell types or after large doses of radiation cell progression is minimal and degeneration begins soon after irradiation, so that the cell never reaches the first mitosis. This mode of radiation lethality is referred to as interphase death. Interphase cell necrosis can be caused in any kind of cell and is unlimited in extent, but usually requires much larger doses than the production of an equal extent of mitosis-linked death, that is doses in the hundreds or thousands of rads. Exceptions are a few cell types such as small lymphocytes, oocytes and spermatogonia that undergo interphase death after low doses of radiation, in the order of one hundred rads or less. (162)

Radiosensitivity

All mammalian cells can be altered in various ways and to various degrees by ionizing radiation. Indeed all living matter can be killed by radiation. The dose required to produce the different effects or similar degrees of the same effect in different types of cells may vary greatly. (159,162) The concept of radiation sensitivity and radiation resistence are relative and are concerned with differences in the degree of responsiveness of cells of different kinds, in terms of some defined effect, when they are exposed to a given amount of irradiation or with the differences in the amount of radiation necessary to cause certain degrees of a specified response. (162)

The ideas and conclusions of early investigators concerning the radiosensitivity among cell types were summarized in 1906 by Bergonie and Tribondeau. On the basis of their studies on irradiated testes they presented three laws which state the radiation acts more intensely on cells when: the reproductive capacity of the cell is greatest; the mitotic process is prolonged; and the differentiation and function are less definitely established or fixed. (162,163) Although this statement has become subject to various interpretations it is generally and freely interpreted to mean that actively proliferating cells are the most sensitive to radiation and that the radiosensitivety of cells varies inversely with the degree of differentiation.

The above principles are still roughly compatible with much of what is known concerning the relative radiosensitivity of different classes of cells. The radiosensitivity of various mammalian cells decreases in the following order: lymphoblasts and lymphocytes, thymocytes, granulocytes (bone marrow and peripheral blood), germ cells (germinal epithelium in testes and oocytes in ovaries), epithelium (gastrointestinal tract), endothelium, muscle, connective tissue, bone and nerve cells. Warren proposed that tumors may be classified as radiosensitive, radioresponsive and radioresistant when a decrease in size follows exposure to twenty-five hundred rads, twenty-five hundred to five thousand rads, and over five thousand rads, respectively. (165)

While the term 'radiosensitivity' appears to be universally accepted and adopted by most authors many recent investigators are not satisfied with the term. (159,161,162,165) The term refers to the radiosensitivity of the process of cell division rather than the functional integrity of the cells in the irradiated tissue. The implication is that cells that do not divide are radioresistant. The authors dissatisfied with the term radiosensitivity argue that all cells are rediosensitive if a dose of sufficient strength is administered. Alternative words such as radioresponsive or radiovulnerable have been proposed.

Additional factors influencing radiation effect

In considering the radiosensitivity, radioresponsiveness or radiovulnerability of a cell or tissue other factors must be considered. These include physical, environmental and biological factors.

The physical factors influencing radiation effects on cells include the total dose, the ionization density or linear energy transfer (LET) of the radiation and the time span over which a total dose of radiation is adsorbed. For a given dose rate and a given observation period after irradiation the degree of histopathological and functional damage to a tissue or organ usually varies directly with size of the total dose.

The linear energy transfer defines the amount of energy transferred per unit path length and involves such considerations as mass, charge and velocity. The LET value of a form of radiation indicates the liklehood of its having an effect on a target area. High LET radiation is more damaging dose for dose, that is, it will have greater relative biological effectiveness (RBE) than low LET radiation. Gamma rays have a relatively low LET because of their penetrability, thus depositing their energy over a long distance. Alpha particles because of their large mass and high charge have a much higher LET. The time span over which a given dose of radiation is absorbed plays an important role in determining its biological action. For a given total dose the degree of histopathological damage to a tissue or organ is usually greater than when it is considerably fractionated or partitioned in time. It has been believed that radiant energy was totally cummulative and had an additive effect. At very high dose rates this concept is basically correct. At relatively low doses cells may recover from their radiant injury and repair within the time span between exposures. If recovery is not complete at the time of the second exposure there will be an additive effect. However the total impact of the radiant energy is not as great as would have been achieved by one single dose equivalent to the sum of the individual doses. There is some evidence that fractionated doses of radiation accelerate repair of cells and that some acquired resistance or adaptation may occur within exposed but still vital cells. (159,162)

The environmental factor that has received the most attention is oxygen tension. An almost three-fold increase in response has been demonstrated under conditions of high oxygen tension as compared with anaerobic conditions when radiation of a low LET has been used. This ability of oxygen to potentiate the effects of ionizing radiation is known as the 'oxygen effect' and has been observed in both invitro and invivo systems. Molecular oxygen has two unpaired electrons. It may therefore interact with radiation induced free radicals to increase the number of 'hot radicals'. Radiosensitivity of cells invitro has also been enhanced by an increase in temperature. (159,162,166)

Biological considerations include differences in radioresponsiveness between individuals and between species. Rats for instance are radioresistant while guinea pigs are radiosensitive. Swine, goats and man fall somewhere in between. (165) The response in invivo systems, particularly latent changes, must take into consideration the consequences of radiation induced injury to vascular and supporting elements of tissues. If the radiation response is high enough to produce vascular changes leading to narrowing or occlusion of small blood vessels, there may be delay in replacement of parenchymal cells dependent on an intact blood supply. In addition changes initiated in the vascular or connective tissue are often not repaired in a typical fashion and this damage may actually increase in severity in the months and years following radiation. These changes are associated with ischemia and fibrosis in the tissue. Therefore after radiation there may be secondary changes in parenchymal cells which are a result primarily of the vascular damage. It is difficult to assess the indirect contribution of these changes following massive radiation exposure. (163)

EFFECTS OF RADIATION ON THE PANCREAS

The exocrine and endocrine cells of the pancreas behave as long-lived reverting post-mitotic cells, that is they divide infrequently but are capable of proliferating on demand. (167,168) There has been relatively little experimental investigation of the pathological effects of radiation on the pancreas. Both the endocrine and exocrine portions of the pancreas are generally considered radioresistant. (167-170)

A series of older studies using the optical microscope described morphological changes in the pancreas following irradiation. Fisher, Groot and Bachem (171) in 1926 reported their experimental work with four dogs. They resected the head and body of the pancreas leaving the main duct and blood supply to the tail intact. They then exteriorized the tail and exposed it to three, four or five erythema doses (approximately 500 to 2500 rads) and then replaced it in the abdominal cavity. The dogs survived from 14 to 157 days. They reported 'severe hyperglycemia' in all dogs. At necroscopy there was evidence of degeneration and fibrosis of the pancreas. Leven (172) in 1933 implanted radium seeds into the pancreas of dogs delivering a radiation dose of 528 to 1584 millicurie hours. Examination of biopsies taken at various intervals from 47 to 166 days revealed marked fibrosis and atrophy of the pancreas around each seed, especially of the acinar tissue. The 'insular tissue' was reported as appearing normal. Several other early studies are cited by Leven (172) and Volk, Wellman and Lewitan. (173) Ivy, McCarthy and Orndoff in 1924, in studying the effects of roentgen rays on gastric secretion, found a marked fibrous atrophy of the pancreas to one-sixth to one-seventh the normal size following one human erythema dose. Rosenbaum in 1927 irradiated exteriorized chicken pancreas. Regressive changes consisting of hyalinization, vacuolization and nuclear hypochromatosis were shown particularly in the acinar tissue. Islet hypertrophy and focal regeneration of the exocrine pancreas were observed in adjacent non-irradiated tissue. Terbuggen and Heinlein in 1932 reported that radiation of exteriorized rabbit pancreas caused degenerative changes in acinar tissue as well as a lethal reduction in blood sugar, despite the presence of normal islets.

During this early period of investigation there were few functional studies. Orndoff, Farrel and Ivy in 1926 examined enzymatic activity of pancreatic secretion after one erythema dose. They reported a transient increase in enzymatic activity which they attributed to a decrease in the volume of secretion. (174) However, there was interest in the effects of radiation on pancreatic function from a clinical point of view. The work done in this area is reviewed by Rauch and Stenstrom. (175) In 1920 Culler reported two cases of pancreatic fistulas which were permanently closed following X-ray therapy and concluded that radiation was beneficial in treating pancreatic fistulas. Morton and Widger in 1940 reported the use of irradiation with 50 to 100 r in air for a total of 250 to 450 r in the treatment of four cases of acute pancreatitis. It was their impression that repeated small doses of irradiation may have shortened the episodes in cases of acute edematous pancreatitis but had very little effect on one case of acute hemorrhagic pancreatitis. Chisholm and Seibel in 1947 noted that serum amylase levels were less elevated in those dogs receiving radiation therapy following acute pancreatitis produced by injection of bile into the accessory pancreatic duct. They concluded that a single exposure to 90 r would be most benificial in the treatment of acute pancreatitis in dogs, while larger doses or repeated small exposures would probably be deleterious to an already damaged pancreas. In 1950 Miller reported cessation of drainage of a chronic pancreatic fistula subsequent to 2600 r in exposures of 200 r repeated over a nineteen day period. The authors noted that it had been the practice at the University of Minnesota to use individual doses of 150 to 200 r to a total of 450 to 800 r in the treatment of conditions necessitating a temporary decrease of pancreatic function.

Rauch and Stenstrom (175) in 1952 undertook a study to look at the qualitative and quantitative effects of irradiation on pancreatic secretions in dogs. Emphasis was placed on volume, pH and enzyme concentration. The effects of irradiation on the histology of the pancreas was also studied. Pancreatic fistulas were created in five dogs. Doses of 400 to 600 r were then administered to a localized area over the pancreas. Stimulation tests were carried out for periods up to eleven days. On the fifth day an additional 400 to 600 r was administered to two of the dogs and further stimulation studies performed. Their findings were as follows. The volume of pancreatic secretion decreased temporarily. In a few instances there appeared to be a secondary increase in volume above normal. No definite alteration of the alkalinity of the juice was noted. In all instances there was a definite depressive effect on amylase, lipase and trypsin output of the pancreas. In general this effect occurred following a twelve to thirty-six hour delay and the period of depression was limited to a few days in each animal studied. Similar findings were noted with the second exposure to radiation except for a more persistent suppression of trypsin. No histological changes were noted in the pancreas.

One of the first studies to look at sequential morphological changes of the dog pancreas after exposure to high dose radiation was that by Archembeau and coworkers (174) in 1966. They described the morphological changes in the pancreas after 4500 rad to 5000 rad of 250kv X-ray delivered in divided doses to the upper abdomen over a period of one month. The pancreas of nine dogs was surgically isolated to the right side of the abdomen prior irradiation. At the completion of irradiation and at one, two, and five months after irradiation histological examination demonstrated progressive interstitial fibrosis and scarring which distorted the regular architecture. The acini remained morphologically intact. The islets showed no histological change. There was no change in fasting blood sugars measured during and before radiation. The functional response of a single dog prepared with a pancreaticoduodenal fistula, prior to, during, and two weeks after 4500 rad delivered over a period of three weeks was also summarized. The secretory volume, stimulated and unstimulated did not change. The serum amylase was initially elevated and then decreased. Amylase activity in the pancreatic secretion increased. The authors concluded that the dog pancreas showed few morphological and functional changes after high dose fractionated radiation. They felt that the changes seen were predominantly the result of progressive vascular damage rather than direct parenchymal cell radiation injury and death.

The work done by Volk, Wellman and Lewitan (173) in 1966 represents the first availabel reference dealing with the untrastructural changes in the pancreas after irradiation. They exteriorized the pancreas of 32 dogs and selectively irradiated the organ in 23 of the dogs with 5000 to 9000 rads. A 250 kv machine was used which delivered 151 rad per minute. Aminals were sacrificed at 30 minutes and 1,2,5,7,8,12,14 and 21 days after irradiation. Pancreatic tissue was sampled and examined by light microscopy, electron microscopy and submitted for amylase, lipase and leucine aminopeptidase determinations. With light microscopy the pancreas showed well granulated beta cells and normal acinar cells. Animals sacrificed at 21 days displayed multiple foci of moderate interstitial as well as perilobular fibrosis. With electron microscopy the acinar cells exhibited large numbers of membrane bound cytoplasmic lesions with varied patterns. These contained altered cytoplasmic components as a variety of pleomorphic electron dense structures consisting of osmophilic agranular membranes, vacuoles and granules of varying size and shape. Maximum acinar cell damage was seen from five to eight days. Between the twelfth and twenty-first days the number of lesions was reduced although various pleomorphic alterations were still seen. With lapsing time after radiation there was a reduction in the number and size of mature zymogen granules. Simultaneous with the above ultrastructural changes there was a decrease in pancreatic amylase, lipase and leucine aminopeptidase. The decrease in enzymatic activity occurred almost immediately post-irradiation. Islets exhibited moderate degranulation, swelling of mitochondria, disruption of endoplasmic reticulum and appearance of electron dense bodies in a number of beta cells. Several alpha cells demonstrated cystic dilatation of the endoplasmic reticulum, deposition of a finely granular intracytoplasmic material and swelling of the mitochondria. In contrast to the acinar cells the alterations in the islet cells became more conspicuous at the end of the experimental period. Despite these changes daily blood sugar levels and glucose tolerance tests maintained normal levels. The authors commented on the pathogenesis of the changes described. The heterogeneous lesions seen in the exocrine cells were similar to structures observed in the pancreas of various species after the administration of DL-ethionine, B3TA, protein deficient diets,and following treatment with pilocarpine and neutral red. They stated that the decrease in enzymatic activity was a result of an inhibition of protein synthesis induced by radiation.

In an extension of the above study, the same authors (176) described three distinct phases of radiation injury to the pancreas. The first was a degenerative phase covering the first few weeks and extending well into the second and third post-irradiation months. This was followed by a recovery phase beginning during the third week but reaching its peak during the third and fourth post-irradiation months. A normalization of the ultrastructural pattern of the acinar cells occurred during the third phase from six to nine months after injury. Despite the morphological normalization they noted a persistent enzymatic depression.

The number of recent studies of radiation injury to the pancreas, again, are few in number. Pieroni and coworkers (177) studied radiation injury to the canine pancreas in six dogs. After control secretory, histological and pancreaticographic studies,six Thomas fistula dogs were subjected to a tumor dose of 400 rad delivered in six sessions on alternate days to a total of 2400 rads. Exocrine secretion demonstrated a consistent pattern in five of the dogs. There was an initial increase in volume of secretion followed by a progressive reduction in volume, bicarbonate and enzyme output (greater than ninety percent after three months). Laparotomy and pancreatic biopsy were performed at seven and eight weeks in three dogs. The pancreas had markedly decreased in size and was nodular and hard. Histologically there was evidence of diffuse interstitial fibrosis with a marked reduction of acinar tissue. There was no change in fasting blood sugar or glucose tolerance tests during the study.

The response of the exocrine pancreas to local irradiation by intraductal injection of radioisotope has been examined in rodent (178) and canine (179) pancreas. A significant and rapid reduction in pancreatic juice enzyme concentration was seen in animals treated with active ebrium compared to animals treated with inactive ebrium or without injection. In the rodent studies endocrine function was not affected at eight months. In the canine model repeated glucose tolerance tests revealed no impairment of endocrine secretion over a five month period of study.

Recently Zook and coworkers (180) undertook a study to determine the relative biological effectiveness of fast neutrons given in a clinical fractionated schedule on the normal canine lung. The report described the pathological effects of fast neutrons and photons on the pancreas, pylorus and duodenum which were included within the radiation field. The dose ranged from 1000 to 6750 rads fractionated over a period of six weeks. Pancreatic lesions included fibrosis and atrophy of the gland with degranulation and necrosis of acinar cells. Islet cells were not obviously damaged containing a full complement of insulin, glucagon and somatostatin granules.

Two studies have suggested that alpha cells are more sensitive to radiation than beta cells. Spalding and Lushbough (181) working with mice, rats and monkeys described necrosis of alpha cells after a single exposure to 2500 r. More than 5000 r was required to produce similar changes in the beta cells. Sejanov and Guljaev (182) working with rats receiving a total body dose of 70 rads, observed death of alpha cells.

Sommers (170) comments on two studies demonstrating radiation induced islet cell tumors in rats. After 430 rads with or without added 3-methylcholanthrene, rats had a fifty-one percent survival rate of fifteen months. In twenty-three males and five females pancreatic islet cell tumors developed. Two islet cell tumors were of alpha or delta cell types. The chemical carcinogen was not implicated. Parabiotic rats with one irradiated partner also formed islet-cell tumors.

The studies reviewed support the following conclusions concerning radiation injury to the pancreas. The exocrine and endocrine pancreas are relatively radioresistent to doses and dosage schedules of radiation used clinically. With high doses of radiation injury does occur. Acinar cells undergo degranulation, necrosis and atrophy. Transient initial hypersecretion of pancreatic enzymes is followed by prolonged and severe reduction of pancreatic secretion. Islet cells may undergo initial degranulation but are otherwise minimally damaged. Alpha cells may be more radiosensitive than beta cells. Islet cell injury, when present, does not result in alteration of blood glucose, glucose tolerance or signs of diabetes mellitus. It is generally agreed that more investigation is necessary to establish precisely the position of the pancreas and its component parts on the scale of radiosensitivity of tissues. Casarett (168) concluded that the studies done have not been definitive or sufficient enough to induce dose-response relationships or provide a histopathological sequence of events.

RADIATION AND PANCREATIC TRANSPLANTATION

Radiation has been applied to the field of pancreatic transplantation by several investigators. It has been used as a means of immunoalteration and as a method to depress exocrine secretion in immediately vascularized pancreas grafts.

Merkel and associates (183) reported their experience with 30 dogs who received whole organ allografts from donors pretreated with 500 rads to the upper abdomen three to seven days prior surgery. Only two dogs developed septic complications. It was their clinical impression that radiation was responsible for the low rate of complications. Kyriakides and coworkers (184,185) assessed several protocols to inhibit autodigestion of heterotopic segmental autotransplants. The protocols included radiation, radiation and steroids,Trasylol and glucagon. In a preliminary report of their findings (184) the gland was best preserved in the group receiving radiation and steroids. In the second report (185) radiation failed to prevent inflammatory and necrotic changes. The best results were seen with glucagon. Tersigni and coworkers (186) irradiated duct-ligated pancreatic allografts in dogs. Two groups of control animals survived five and six days post-transplantation. Animals received grafts treated with 1000 rad, 5000 rad and 10000 rad. The survival was 19 days, 27.5 days and 39.5 days respectively. At the time of necroscopy the pancreas in control animals showed significant inflammatory changes with hemorrhagic necrosis of the gland. In the irradiated group, the predominant finding was fibrosis and lymphocyte infiltration. Faure and coworkers (179) reported their experience with local irradiation of segmental autografts by intraductal injection of ebrium-169. Glucose peripheral disposal (K value) at six months demonstrated a mean reduction of 28 percent in an irradiated group versus 40 percent in a nonirradiated control group when compared with the preoperative values in the same dogs.

There are two reports of the use of radiation in clinical whole organ transplantation. Kelly and associates (187) reported the case histories of two recipients of pancreatico-duodenal allografts. Low doses of radiation (150 r for several days)were used post-transplant to circumvent rejection. Allograft survival was prolonged in one of the patients. Tersigni and associates (188) reported the results of three irradiated and duct-ligated segmental pancreatic autotransplants in patients with cancer of the pancreatic head. In the first patient the graft was treated with 5000 rads. The necrotic graft was removed on the fifteenth postoperative day. In the second and third patient the graft received 2000 rads. Functioning grafts without exocrine secretion and with normal carbohydrate metabolism were reported at seven months in the second patient, and at one month in the third patient.

Lacy and associates (189) appear to be the only investigators who have applied radiation to the field of pancreatic islet cell transplantation. Pretreatment of donor rats with a total body dose of 850 rads and silica followed by invitro culture of the islets for one to two days prolonged survival of allografts across a minor histocompatibility barrier if 'hand-picked' clean islets were used for transplantation. A single injection of antilymphocyte serum was necessary to prolong survival across a major histocompatibility barrier. Controls within the study established that the effects of radiation was independent of that of silica. The period of tissue culture and hand-picked islets were necessary to prolong allograft survival.

VI STATEMENT OF HYPOTHESIS

A practical and efficient means of separating the endocrine components of the pancreas from contaminating exocrine cells, as well as ductal, vascular endothelial, and immune cells is required to overcome the barriers to clinical islet cell transplantation. The objective of this study was to use high dose radiation to selectively injure contaminating exocrine cells, leave endocrine cells viable and purify a graft preparation of pancreatic fragments. To meet this objective it was necessary to evaluate two major considerations or questions.

Question 1: The literature available on radiation injury in the pancreas suggested that high doses of radiation administered to the intact and vascularized gland could selectively injure exocrine cells and leave endocrine cells viable. The first question to be answered in this study was whether or not similar findings could be observed when radiation in high doses was delivered to a suspension of pancreatic fragments. To answer this question it was proposed to deliver a low, moderately high, and high dose of radiation to a graft of pancreatic fragments prepared from one pancreas and compare the response from each dose of radiation to a control from the same pancreas.

Question 2: Methods presently being used or investigated to purify graft preparations of pancreatic fragments, such as tissue culture are inefficient. Insulin secreting tissue is lost during the purification process. As a consequence of this low islet recovery autotransplants of purified islets in the canine model have not been uniformly successful. The second consideration in this study was to assess the recovery of viable islets after purifying a graft with radiation (pending demonstration that the graft could in fact be purified with radiation). To do this it was proposed to compare survival and metabolic function of a control series of dogs receiving an intrasplenic autograft of untreated pancreatic fragments to a series of dogs receiving an intrasplenic autograft of pancreatic fragments treated with a dose of radiation necessary to purify the graft.

VII MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Invitro Studies (Figure 1)

A graft preparation (n=8) of partially purified pancreatic microfragments prepared from 8 adult canine pancreases was equally divided into 4 groups. Group I served as a control and the remaining groups were treated with increasing doses of radiation, single fraction: group II - 2500 rad; III - 5000 rad; IV - 7500 rad. Amylase activity was measured in the supernatant of the graft immediately (within 30 min.) following radiation. Purification and islet recovery were assessed by measuring the insulin and amylase concentrations of the pancreas (n=7), processed graft (n=7), and groups I-IV immediately following radiation (n=8) and after 8h (n=6) and 24h (n=6) of tissue culture. These measurements were made on the assumption that the insulin and amylase content of the graft (per unit weight of tissue) are proportional to islet cell mass and exocrine enzyme content of the graft respectively and that the ratio of insulin to amylase (I/A) is an index of graft purity (85). Tissue sampled at the same intervals was fixed for electron microscopy to evaluate ultrastructural changes in the irradiated graft. Invitro viability of beta cells following radiation was assessed by stimulation with glucose in perifusion. Viable beta cells respond with a typical biphasic release of insulin (190, 191).

EXPERIMENTAL DESIGN

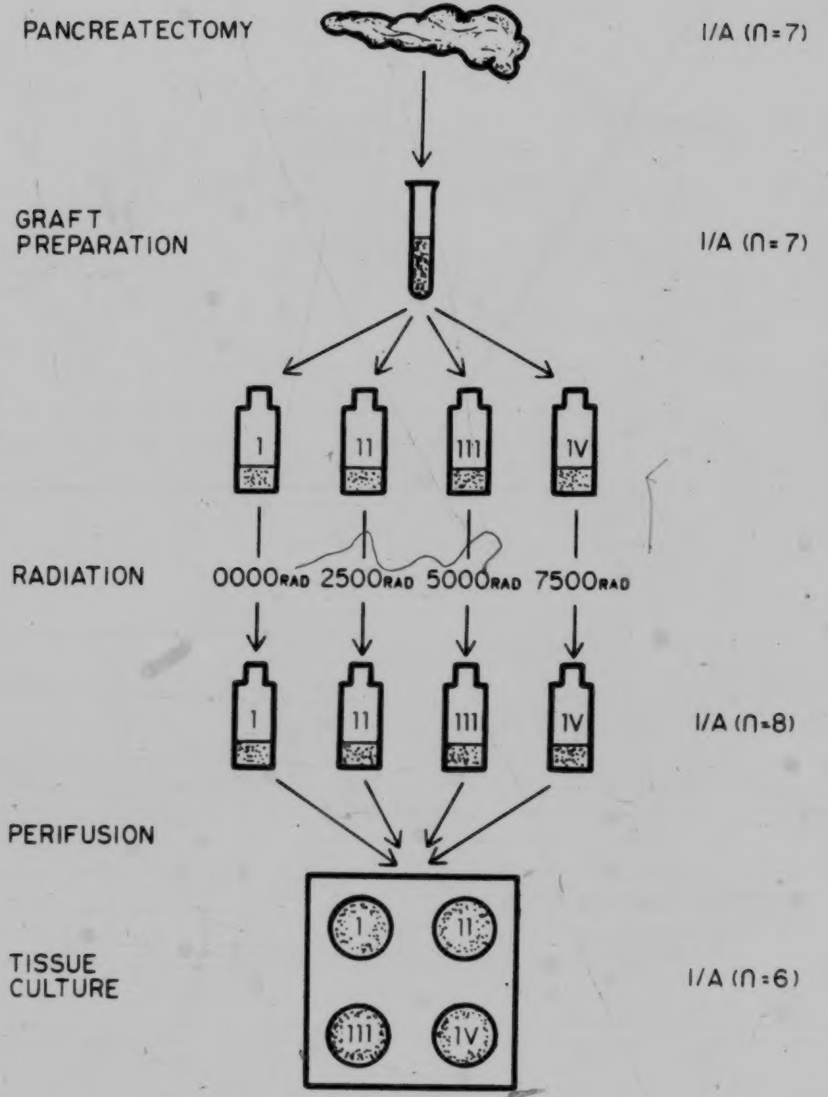

PANCREATECTOMY
I/A (n=7)
GRAFT PREPARATION
I/A (n=7)
I
II
III
IV
RADIATION
0000RAD
2500RAD
5000RAD
7500RAD
I
II
III
IV
I/A (n=8)
PERIFUSION
TISSUE CULTURE
I
II
III
IV
I/A (n=6)

FIGURE 1. Experimental design - Invitro studies. The graft was divided into four groups - I, the control, and groups II-IV irradiated. Islet recovery and purification assessed by comparing the insulin/amylase (I/A) ratio of the pancreas, graft, and groups I-IV following radiation and after 8h and 24h of tissue culture. Viability of endocrine cells following radiation assessed by glucose challenge in perifusion.

Invivo Studies (Figure 2)

The first 5 dogs undergoing total pancreatectomy for the invitro studies were maintained as apancreatic controls. In 16 dogs total pancreatectomy was performed one week after an intravenous glucose tolerance test (ivGTT). Nine dogs, group Ia, received an intrasplenic autotransplant of fresh graft immediately following graft preparation. Seven dogs, group IIa, received an intrasplenic autotransplant of graft after invitro radiation of the graft with 5000 rad, single fraction. It was proposed to determine fasting serum glucose on day 1, 3 and 6 post-transplant and then weekly for one month and perform ivGTT at one month post-transplant to compare autograft function and survival in groups Ia and IIa.

To determine a uniform end-point for comparison of survival dogs were sacrificed when they lost 25% of preoperative weight in face of hyperglycemia.

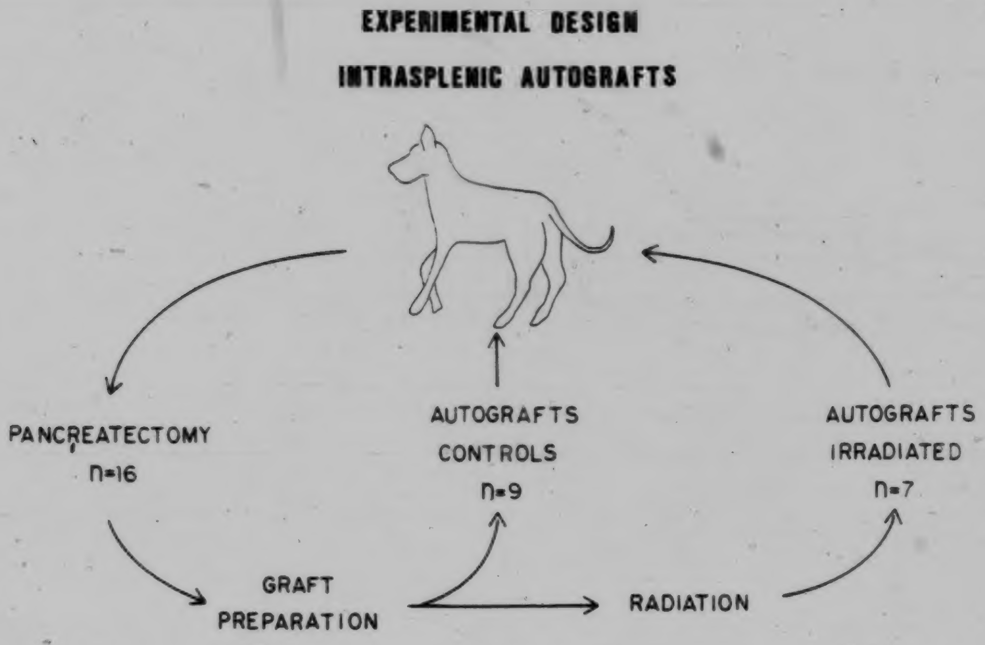

EXPERIMENTAL DESIGN
INTRASPLENIC AUTOGRAFTS
PANCREATECTOMY
n=16
GRAFT
PREPARATION
AUTOGRAFTS
CONTROLS
n=9
RADIATION
AUTOGRAFTS
IRRADIATED
n=7

Figure 2. Experimental design - Invivo studies. Autograft function and survival at one month after transplant compared between apancreatic dogs receiving intrasplenic autografts of untreated graft (control group Ia) and graft treated with 5000 rad (irradiated group IIa).

ANIMALS AND CARE

Twenty-four mongrel dogs of both sexes (mean age 2.8 years, range 1-5 years) weighing 15-27.5kg were studied (Table 1). They were housed in the large animal vivarium of the Surgical-Medical Research Institute, University of Alberta, under care of the investigator with advice from a veterinarian in accordance with criteria formulated by the Canadian Council on Animal Care. Surgical procedures were carried out under general anesthesia with sodium pentabarbitol, 30mg/kg/body weight. The dogs were weighed weekly and permitted unrestricted exercise twice a day. Full diet consisted of 360g of meat (Dr. Ballard's) and 600g of Burger Bits (Pow R Pac) daily. On postoperative day 1 hydration was maintained with 1L of normal saline injected subcutaneously. On postoperative day 2 water was allowed, on day 3 a meat diet was offered and on day 4 full diet resumed. Each meal was supplemented with 8 capsules of Cotazym (Organon, Montreal). Prophylactic antibiotics, Derapen-C (Ayerst, Montreal) 3-4ml, were administered at the onset of surgery, immediately postoperatively, and on postoperative day 1.

Table 1. Vital statistics on dogs used in study.

EXPERIMENTAL Group	ID	AGE (YR)	SEX	WEIGHT (kg)	DATE OF SURGERY	SYMBOL*
Invitro Studies	E146	2	M	24.0	12/09/84	○
	E147	1	M	15.0	26/09/84	⬡
	E230	4	M	24.0	30/10/84	□
	E293	2	F	19.0	06/11/84	△
	E283	2	F	20.0	14/11/84	●
	E339	4	F	24.0	20/02/84	⬢
	E421	5	M	24.0	20/02/84	■
	E436	5	M	24.0	20/03/84	▲
Control Autografts (Group Ia)	E342	1	M	22.5	18/01/85	
	E335	5	M	25.0	01/02/85	
	E388	3	M	23.0	22/02/85	
	E413	3	M	22.5	28/02/85	
	E449	1	M	20.5	07/03/85	●
	E410	2	M	25.5	27/03/85	
	E456	2	M	22.0	28/03/85	
	E470	4	F	25.5	02/05/85	
	E459	2	M	25.0	04/05/85	
Irradiated Autografts (Group IIa)	E368	1	M	18.0	05/02/85	
	E418	1	M	24.5	26/02/85	
	E422	4	M	25.0	05/03/85	
	E437	4	M	27.5	12/03/85	■
	E452	2	M	23.0	26/03/85	
	E478	4	M	23.0	16/04/85	
	E495	2	M	21.0	23/04/85	

*Symbol used to represent individual dogs in invitro studies and groups of dogs in invivo studies for graphical display of results.

PANCREATECTOMY

The dogs were anesthesized with sodium pentabarbitol (30mg/kg) then intubated and intravenous administration of normal saline started at a rate of 150ml per hour. The pancreatectomy was performed as described in detail by Cobb and Merrel (192). Through an upper midline incision the entire pancreas was mobilized with the four major vascular connections preserved. Both branches of the pancreatic duct were cannulated (20-22 guage polyethylene tubing) while the gland was insitu. A 0.2g sample of pancreas was excised for insulin and amylase assay with care being taken to ligate the pedicle from which it was removed. Finally, the blood vessels were clamped, ligated, and the gland removed. Immediately the pancreas was weighed and ducts distended by injection of Hank's balanced salt solution (HBSS) approximately 60ml into the horizontal limb (body and tail) and 20ml into the vertical limb (uncinate process). The gland was immersed in HBSS at 4°C and transferred to the laboratory for graft preparation. In the invitro studies the abdomen was closed in the 5 dogs maintained as apancreatic controls. The other 3 dogs were sacrificed by lethal injection at completion of pancreatectomy. In the invivo studies an assistant maintained the 16 dogs under general anesthesia during graft preparation (group Ia - 9 dogs) and during graft preparation and irradiation of the graft (group IIa - 7 dogs).

GRAFT PREPARATION

The graft was prepared by a method previously reported from this lab by Warnock (22), a modification of the technique initially described by Horaquchi and Merrel (95) (Figure 3). The cannulas, placed intraoperatively in the two major ducts, were perfused with chilled HBSS at 10-20ml per minute for 12 min. The perfusate was then changed to a solution of 0.4% collagenase (Type V, 250 U/mg, Sigma Chemicals, St. Louis, Missouri) in HBSS at 37°C. The flow was in a retrograde direction through ducts of decreasing size distending the acini and rupturing tissue within the pancreatic lobules. The gland was not immersed in collagenase. It was suspended on a 60-um mesh above the reservoir of collagenase from which the enzyme was reperfused. When the gland was mushy or mucoid (average 30 min.) digestion was judged complete. The digested gland was bisected and transferred to two mincing jars containing 50ml of enzyme-stop solution consisting of 4°C HBSS supplemented with 2% Trasylol (vol/vol; Miles, Pharmaceuticals, Elkart, Indiana), penicillin 100U/ml and streptomycin 100 ug/ml. The tissue was minced for exactly 90 seconds in a mechanical mincer (141). The supernatant fluid was discarded and the preparation resuspended in fresh enzyme-stop solution and transferred to an Erlenmyer flask. Dissociation was accomplished by shaking the flask vigorously in an Evapomix for 10 min. at 4°C, followed by filtration through a 400-um screen. Residual unfiltered tissue was returned to the Erlenmeyer flask and passed through the dissociation process again. The residual tissue was not further digested with trypsin as described by Horaguchi and Merrel (95). Finally, the preparation was washed x3 in enzyme-stop solution. The filtrate was centrifuged at 400g for 20 seconds and supernatant fluid removed. The pellet of islet tissue was resuspended in 30-40cc of enzyme-stop solution prior to radiation in the invitro studies (groups I-IV) and prior to immediate autotransplantation (group Ia). Prior to radiation in the invivo studies (group IIa) the pellet was resuspended in 150ml of tissue culture medium RPMI 1640, (Gibco Laboratories, New York, catalogue no. 380-2400) supplemented with 2% Trasylol (vol/vol), 10% foetal calf serum, penicillin 100U/ml and streptomycin 100ug/ml. Attention was paid to aseptic technique with the majority of the procedure performed under the laminar flow hood. The time for graft preparation was approximately 2h.

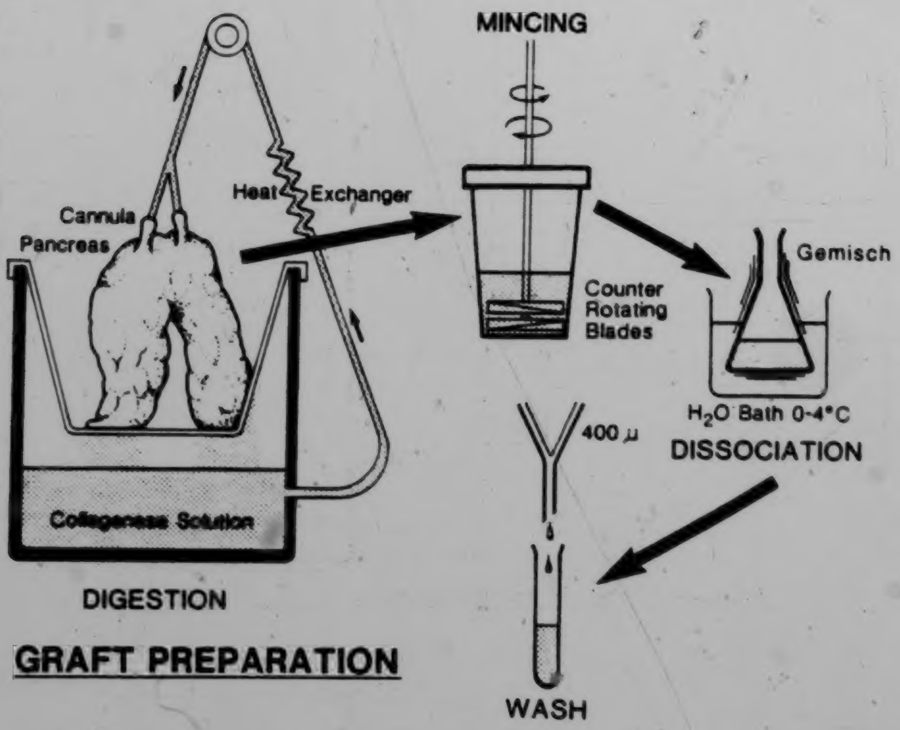

MINCING
Heat Exchanger
Cannula
Pancreas
Counter Rotating Blades
Gemisch
H_2O Bath 0-4°C
DISSOCIATION
400 µ
Collagenase Solution
DIGESTION
GRAFT PREPARATION
WASH

Figure 3. Steps included in graft preparation: digestion, mincing, dissosciation, washing, to prepare a suspension of islet containing fragments.

RADIATION

The graft was irradiated at the W. W. Cross Cancer Institute, Edmonton, Alberta (Figure 4). The pancreatic microfragments contained in tissue culture flasks (Falcon No. 3013, Beckam and Dickinson, Oxnord California in groups I-IV; Falcon No. 3024 in group IIa) were transported in an ice bath. The culture flasks were placed in a jig made of plexiglass containg ice which sat within the housing of the Theratron 780 (Atomic Energy of Canada Ltd.). The source of radiation was cobalt 60. Experimental calibration was done by determining the dose of radiation adsorbed by lithium powder which was spread on the bottom of the flasks. Appropriate adjustments were made for the fluid volume (10cc) in each flask. With the collimators of the Theratron 780 wide open an average dose of 615 rad/min. was delivered to the contents of each flask. The error in the calculation was approximately 3%. To deliver an accurate dose to the pancreatic fragments in groups II-IV each flask was left in the plastic jig and exposed to radiation for an appropriate time interval. For example, in the invitro studies group II was removed from the plastic jig after 4.06 minutes having received 2500 rad and placed back in the ice bath with the control, group I. After 8.12 seconds group III was removed from the jig, having received 5000 rad and placed back in the ice bath with groups I and II and so on. In the invivo studies, group IIa, the one flask was exposed to radiation for 8.12 seconds to deliver a dose of 5000 rads to the contained graft. As the experiment progressed appropriate adjustments were made in the time intervals to correct for decay of the cobalt 60 source, approximately 1% reduction in dose per month. The procedure including transport back to the Surgical-Medical Research Institute took an average of 1h.

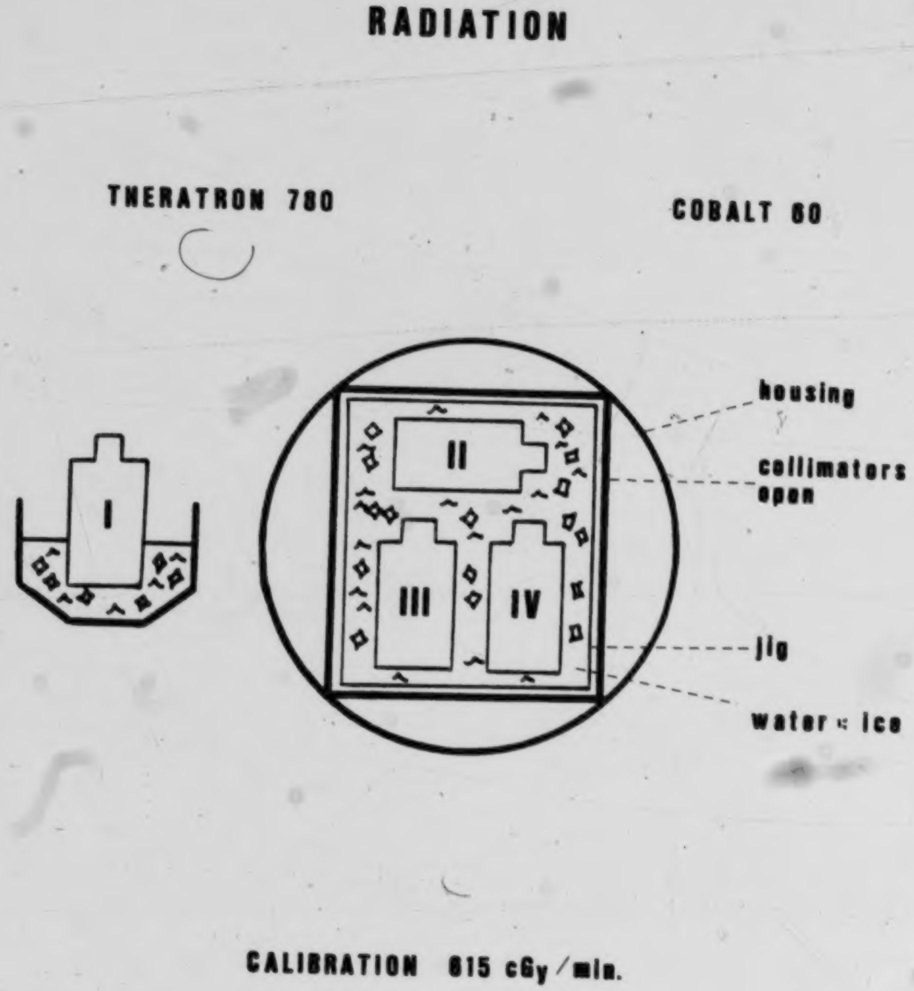

RADIATION
THERATRON 780
COBALT 60
I
II
III
IV
housing
collimators open
jig
water & ice
CALIBRATION 615 cGy/min.

Figure 4. Irradiation of pancreatic fragments. Groups II-IV placed in jig containg ice within housing of Theratron 780. With collimators wide open a dose of 615rad/min. was delivered to the content of each flask. Desired dose delivered to each group by removing each flask from the jig after an appropriate length of exposure.

TISSUE CULTURE

Following irradiation the contents of each flask were washed x3 and resuspended in tissue culture medium (TCM), RPMI 1640 supplemented with 2% Trasylol (vol/vol), 10% foetal calf serum, penicillin 100U/ml and streptomycin 100ug/ml, with a glucose content of 150mgm/dl. Approximately 150ml of TCM was used for each 1g of graft. Incubation took place in stationary flasks (Falcon No. 3024) 2 per experimental group, in an atmosphere of room air and 5% CO_2 at 37°C for 24h. TCM was not changed during the culture period. The contents of one flask in each group was sampled at 8h and the contents of the second flask in each group sampled at 24h. The tissue was sampled by gently titrating approximately 80% of the TCM from each flask and resuspending the graft in the remaining TCM by gentle agitation. The suspension of cells was then pipetted into preweighed vials. Each vial was centrifuged for 60 seconds at 400g and the remaining TCM removed. The weight of tissue recovered was recorded and the sample frozen for future insulin and amylase assay.

PERIFUSION

The perifusion apparatus and procedure used was similar in principle, to that described by Lacy (190, 191) (Figure 5). Following irradiation, the contents of each flask (groups I-IV) were washed x3 in TCM. A small aliquot of graft (0.1g) was placed on a 25-um Nitex filter (Tetko, New York) in the perifusion chamber, a Millipore filter unit (Millipore Corp., Bedford, Massachusettes). Perifusion medium RPMI 1640 supplemented with 2% Trasylol (vol/vol), 10% foetal calf serum, penicillin 100U/ml and streptomycin 100ug/ml with a glucose content of 50mg/dl was pumped (Proportioning Pump, Technicon Instruments Co. New

York) through standard i.v. tubing from a reservoir (Erlenmeyer flask) for a 60 min. period of stabilization at a rate of 1ml/min. At 60 min. the glucose content of the perifusion media was increased to a stimulatory level of 500mg/dl for a period of 60 min. At 120 min. and until completion of perifusion at 180 min. the glucose content of the perifusion media was decreased back to 50mg/dl. The effluent from the perifusion chamber was sampled mechanically by a computer driven Autosampler (Technicon Instruments Co., New York). The effluent was collected at 5 min. intervals except for a 10 min. period following initial stimulation when sampling was at 1 min. intervals. After the volume was recorded the samples were frozen for subsequent insulin assay. The apparatus was placed in an incubator and the procedure performed in 95% O_2 and 5% CO_2 at 37°C.

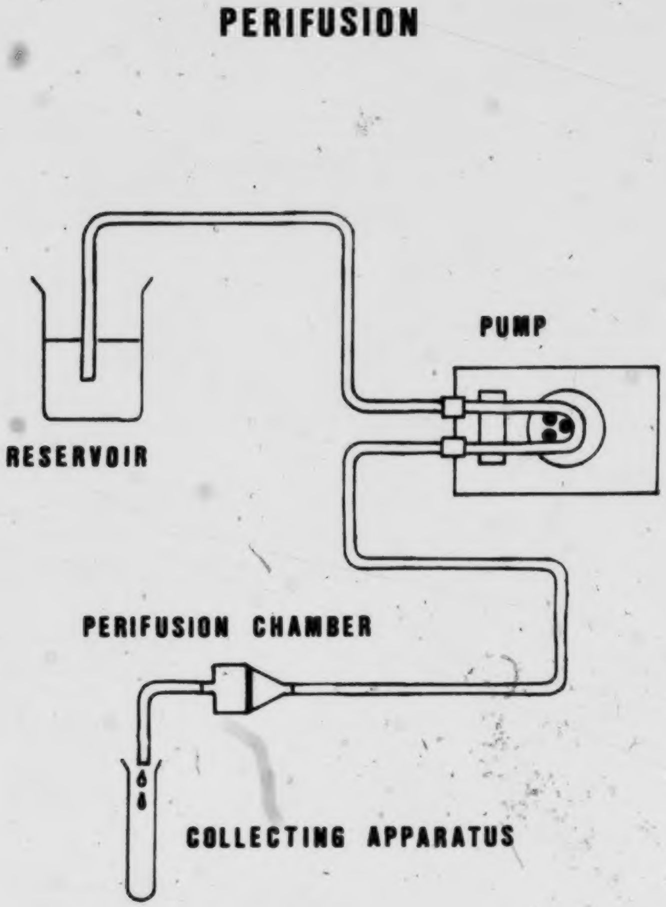

PERIFUSION
PUMP
RESERVOIR
PERIFUSION CHAMBER
COLLECTING APPARATUS

Figure 5. Perifusion. A small sample of graft rests on a filter within the perifusion chamber. The pump pulls medium from the reservoir propelling it through the perifusion chamber. A computer driven auto-sampler (collecting apparatus) samples effluent for the rate of insulin secretion. The entire apparatus is placed in an incubator and procedure performed in an atmosphere of 95% O_2 and 5% CO_2.

ELECTRON MICROSCOPY

Graft was fixed for electron microscopy in 25% (vol/vol) glutaraldehyde followed by 1% (wt/vol) osmium tetroxide. Both fixatives were dissolved in 0.05M phosphate buffer adjusted to ph7.4. After dehydration the fixed tissue was embedded in Araldite CY212 (Marivac, Halifax, Canada). Sections were prepared on the Porter-Blum MT-2 Ultra-Microtome (Sorvalle, Newtowne, Connecticut) picked up on 300-mesh copper grids and stained with uranyl acetate (193) followed by lead citrate (194). The electron microscopy was carried out with a Philips EM-410 (Philips Co., Eindhoven, Holland) transmission electron microscope.

TISSUE ASSAYS

Samples of fresh pancreas, fresh processed graft and irradiated and cultured graft were weighed homogenized and sonicated for insulin and amylase assays. Insulin was extracted after sonication in 5ml of chilled acid: alcohol. After 24h the samples were neutralized and diluted in Delbecco's phosphate buffer solution (Gibco, Grand Island, New York). Insulin was measured by double-antibody radioimmunoassay (195) and amylase (no acid extraction) with Dri-STAT Amylase-DS Reagent (Beckman Instruments, Carlsbad, California) (196).

TRANSPLANTATION PROCEDURE

The graft, resuspended in 30ml of chilled HBSS with 2% Trasylol, penicillin and streptomycin, was isografted immediately following graft preparation in 9 dogs (group Ia) and immediately following graft preparation and radiation in 5 dogs (group IIa). The spleen was delivered into the operative field and 22 guage polyethelyne tubing inserted by venotomy into superior and inferior terminal splenic veins advancing the tubing to the hilus and not into the spleen. Vascular clamps were applied to the splenic pedicle. The graft suspension was injected into the cannulas over a period of 5 min. to allow tissue to reflux into venous sinusoids. The cannulas were then withdrawn and veins ligated. The vascular clamps were released 5 min. after injection of the graft was completed.

BLOOD INDICES

Samples taken preoperatively, during ivGTT, and at the specified times postoperatively were collected in tubes containing heparin and serum removed at once. Fasting serum glucose (BG, mgm/dl) was measured with the Beckman Glucose analyzer (197). Immunoreactive insulin (IRI, uU/ml) was determined by double-antibody radioimmunassay (195) using porcine insulin standards (Wellcome Reagents, Beckenham, England). For ivGTT saphenous veins were cannulated, glucose (0.5g/kg body wt) was injected and blood collected at 0, 1, 5, 10, 15, 30, 60, 90 min. for assays of glucose and insulin. The K value (196), a measure of the decline in glucose level (percent/min.) was determined from glucose levels at 5, 10, 15 and 30 min.

ANALYSIS OF DATA

All results are expressed as mean ± SEM. The data were analyzed with analysis of variance or Student's t-test for paired or unpaired data as applicable. Differences are stated as significant when $P < 0.05$ unless indicated otherwise.

VII RESULTS

INVITRO STUDIES

Amylase Activity - Supernatant (Figure 6)

The amylase activity (IU/L) measured in the supernatant of groups I-IV immediately after radiation showed a consistent response. In 5 of the 6 dogs the amylase activity appeared to decrease in the irradiated groups (II-IV) compared to the control group (1). The mean response followed a similar trend: group I=4,818 ± 1497; II=4,229 ± 1,266; III=3,870 ± 1,041; IV=3,651 ± 1,229. The differences observed in the mean response were not statistically significant. Variability between dogs was statistically significant ($P < 0.01$).

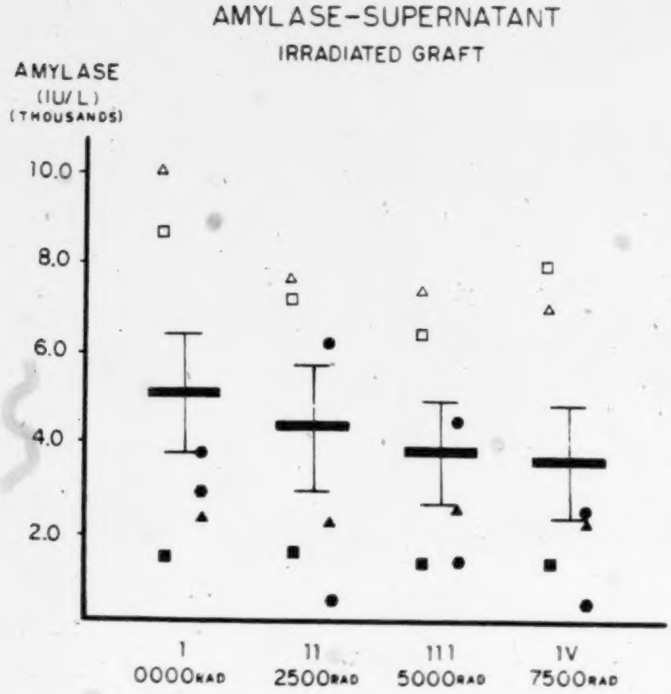


Figure 6. Amylase activity measured in the supernatant of groups I-IV immediately following radiation. Scattergram shows response in individual dogs. Each dog is represented by a different symbol (Table 1). Solid horizontal bars indicate mean and vertical lines the SEM.

Amylase content - Tissue (Figure 7)

The amylase content (IU/g) measured in tissue sampled from the pancreas (P), untreated graft (G) and groups I-V immediately following radiation did not show a consistent response. Relative to the control group (I) the amylase content of groups (II-IV) increased or decreased in different dogs with increasing doses of radiation. The mean response was: P=2,360 ± 441; G=1,149 ± 248; I=1,143 ± 339; II=1,050 ± 301; III=1,096 ± 278; IV=1,117 ± 314. These means were significantly different ($P < 0.01$). The amylase content of the untreated graft and groups I-IV differed significantly from the amylase content of the pancreas ($P < .01$). The marginal decrease in amylase content of the irradiated groups (II-IV) relative to the control group (I) and the untreated graft was not significant. The variability between dogs was significant ($P < 0.01$).

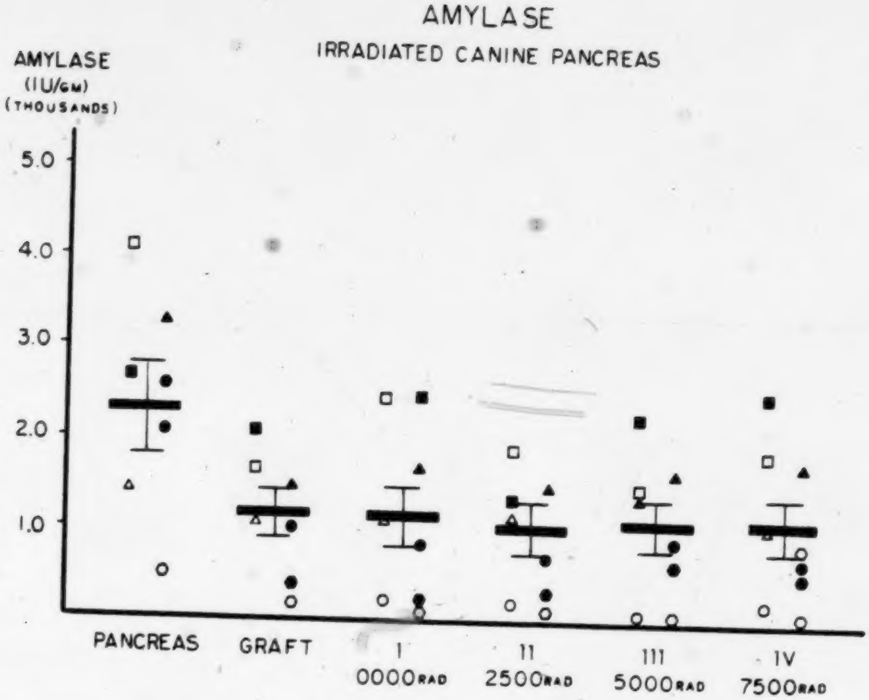


Figure 7. Amylase content of pancreas, untreated graft and groups I-IV immediately following radiation. Scattergram shows response in individual dogs. Each dog is represented by a different symbol (Table 1). Solid horizontal bars indicates mean and vertical lines the SEM.

Insulin Content - Tissue (Figure 8)

The insulin content (uU/gm) measured in tissue sampled from the pancreas, untreated graft and groups I-IV followed a consistent pattern. There was an increase in insulin content in 6 of the 8 dogs in the irradiated groups (II-IV) compared to the control group (I). In 3 of these 6 dogs islet recovery appeared to be the best in group III (5000 rad). In the other 3 islet recovery was best in group IV (7500 rad). The mean response was: P=1466 ± 411; G=1117 ± 273; I=1586 ± 479; II=1543 ± 376; III=2112 ± 423; IV=2151 ± 535. The means were not significantly different. The varability between dogs was significant ($P < 0.01$).

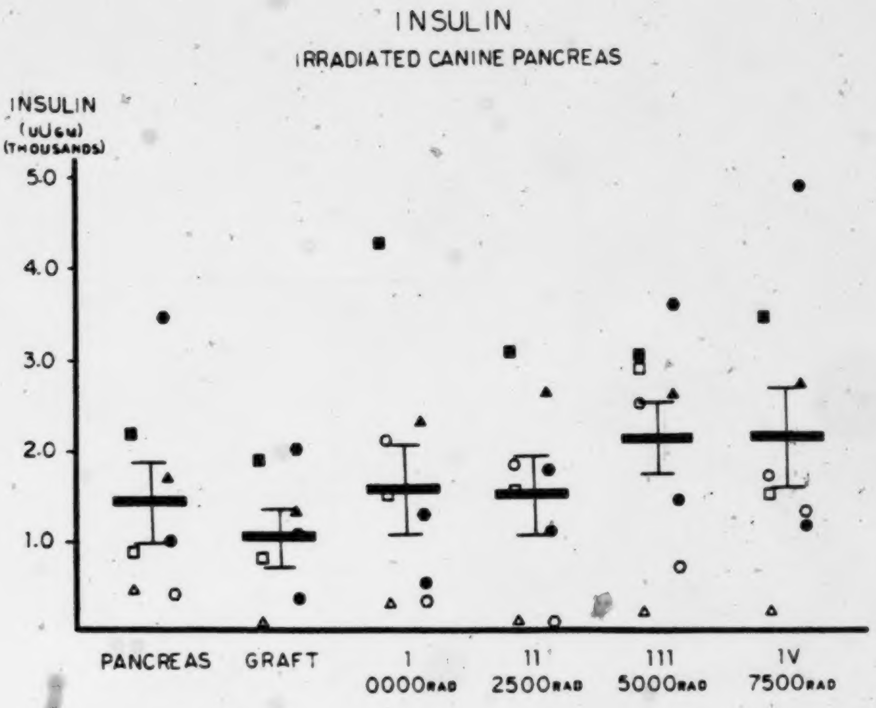


Figure 8. Insulin content of pancreas, graft and groups I-IV immediately following radiation. Scattergram shows response in individual dogs. Each dog is represented by a different symbol (Table 1). Solid horizontal bar indicate mean and vertical lines the SEM.

Insulin/Amylase Ratio (Figure 9)

The insulin/amylase (I/A) ratio followed a consistent pattern with an increase seen in the irradiated groups (II-IV) relative to the control group (I) in 6 of the 8 dogs. The mean response was: P=0.7 ± .01; G=1.8 ± 0.8; I=2.9 ± 1.1; II=2.6 ± 1.1; III=5.2 ± 2.3; IV=4.6 ± 1.9. The means were significantly different ($P < 0.01$). The I/A ratios of groups III and IV differed significantly from the I/A ratio of the pancreas ($P < .001$) and from the I/A ratio of the untreated graft ($P < 0.01$). The differences between groups I and II and the untreated graft were not significant. Variability between dogs was significant ($P < 0.01$).

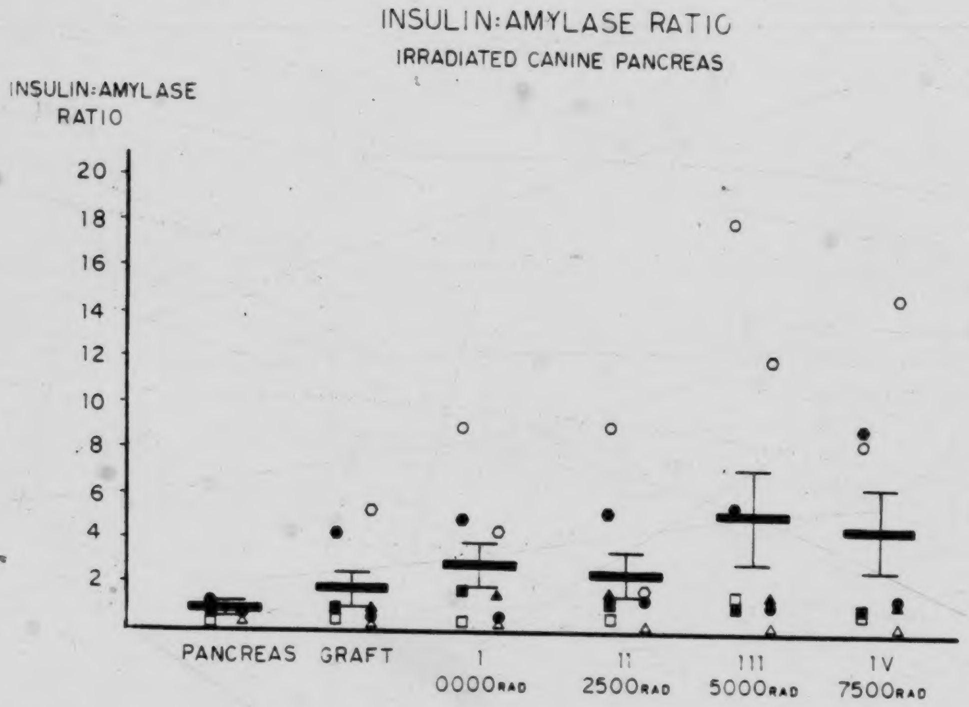


Figure 9. Insulin/amylase ratio of pancreas, graft and groups I-IV immediately following radiation. Scattergram shows response in individual dogs. Each dog is represented by a different symbol (Table 1). Solid horizontal bars indicate mean and vertical lines the SEM.

Tissue Culture

Insulin/amylase ratio: A similar trend was observed in groups I-IV. The I/A ratio in 5 of the 6 dogs increased over the 24h period of tissue culture. The mean I/A ratios from the 6 dogs studied are recorded in Table 2. The differences observed in the mean response between groups I-IV and within each group were not statistically significant. However, compared to the I/A ratio of the untreated graft, a significant increase in the I/A ratio at 8h was seen in group I ($P < 0.05$) and group II ($P < 0.05$) and at 24h in group I ($P < 0.01$), group III ($P < 0.05$) and group IV ($P < 0.05$).

Table 2. Insulin/amylase ratio of graft (groups I-IV) during tissue culture (mean ± SEM).

	I 0000 rad	II 2500 rad	III 5000 rad	IV 7500 rad
Immediately after radiation (n=6)	1.5 ± 0.7	1.8 ± 0.7	2.0 ± 0.7	2.4 ± 1.3
After 8h of tissue culture (n=6)	3.2 ± 1.5	3.8 ± 1.5	3.3 ± 1.8	3.7 ± 1.7
After 24h of tissue culture (n=6)	6.7 ± 3.1	4.3 ± 1.9	6.3 ± 3.2	3.7 ± 1.4

Pancreas (n=6)=0.6 ± 0.2 Graft (n=6)=1.2 ± 0.6

Insulin content of tissue: A similar trend was observed in group I-IV. The insulin content (uU/gm) was maintained in the 6 dogs studied for the first 8h of tissue culture. From 8h to 24h of tissue culture the insulin content decreased in all 6 dogs. The mean insulin content measured after 8h and 24h of tissue culture is recorded in Table 3. The differences observed in the mean response between the groups were not statistically significant. The decrease in insulin content in group II and IV from 8h to 24h of tissue culture was significant ($P < 0.05$).

Table 3. Insulin content (uU/gm) during tissue culture (mean ± SEM).

	I 0000 rad	II 2500 rad	III 5000 rad	IV 2500 rad
Immediately after radiation (n=6)	1711 ± 601	1712 ± 428	2260 ± 522	2349 ± 706
After 8h of tissue culture (n=6)	1983 ± 614	2004 ± 572	1899 ± 614	1735 ± 531
After 24h of tissue culture (n=6)	852 ± 469	716 ± 380	774 ± 463	619 ± 298

Pancreas (n=6)=1634 ± 443

Graft (n=6)=1122 ± 323

Amylase content of tissue: A similar trend was observed in groups I-IV. The anylase content (IU/g) decreased in 5 of the 6 dogs for the first 8h of tissue culture. A further decrease in amylase content occurred in all 6 dogs from 8h to 24h of tissue culture. The mean amylase content measured after 8h and 24h of tissue culture is recorded in Table 4. The differences observed in the mean response between the groups were not statistically significant. The decrease in amylase content over the first 8h of tissue culture was significant in groups I to IV ($P < 0.05$). The decrease in amylase content from 8h to 24h was also significant in groups I and II ($P < 0.05$) and groups III and IV ($P < 0.01$).

Table 4: Amylase content (IU/g) of graft (groups I-IV) during tissue culture (mean ± SEM).

	I 0000 rad	II 2500 rad	III 5000 rad	IV 7500 rad
Immediately after radiation (n=6)	1474 ± 355	1348 ± 312	1426 ± 238	1439 ± 317
After 8h of tissue culture (n=6)	543 ± 106	570 ± 103	719 ± 135	566± 104
After 24h of tissue culture (n=6)	112 ± 47	153 ± 74	95 ± 41	142 ± 48

Pancreas (n=6)= 2668 ± 374

Graft (n=6)= 1308 ± 225

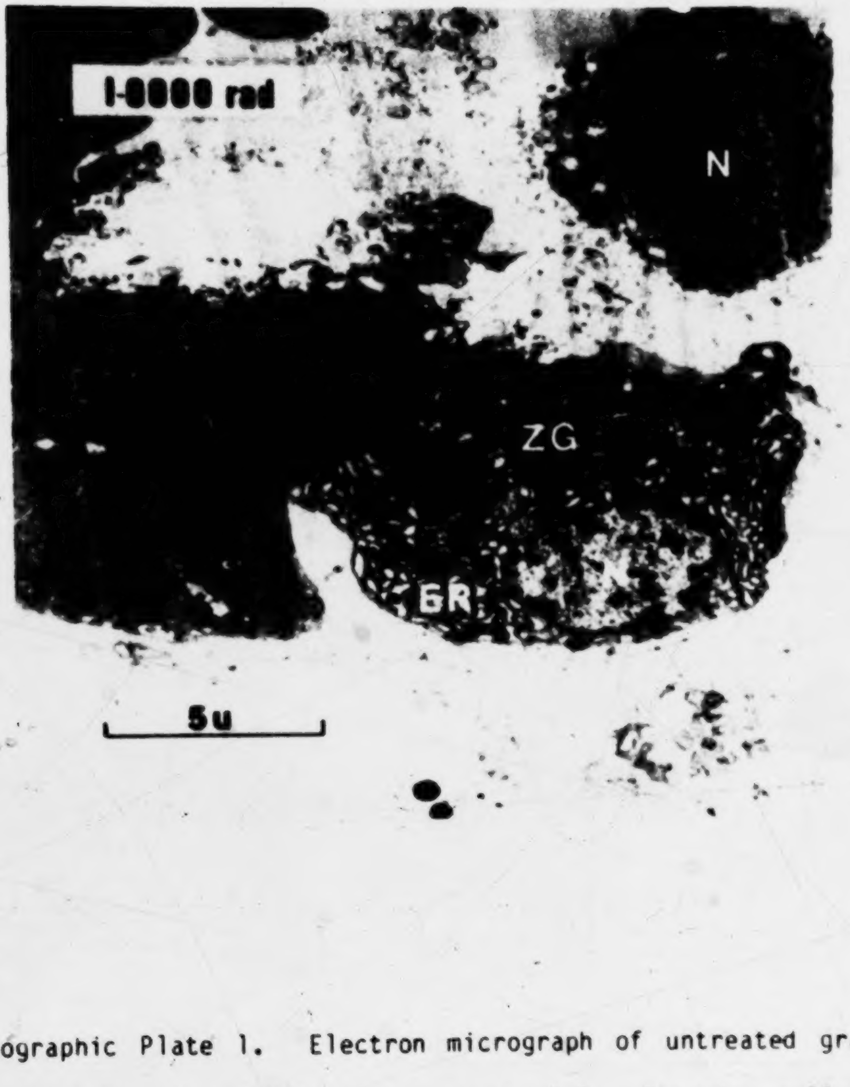
Electron Microscopy

Electron microscopy was reviewed in 6 of the 8 dogs. The findings were similar in each dog. Representative sections from groups I-IV immediately after radiation, from one dog, are reproduced in Photographic Plates 1-4. These photographic plates were reviewed with the assistance of a pathologist.

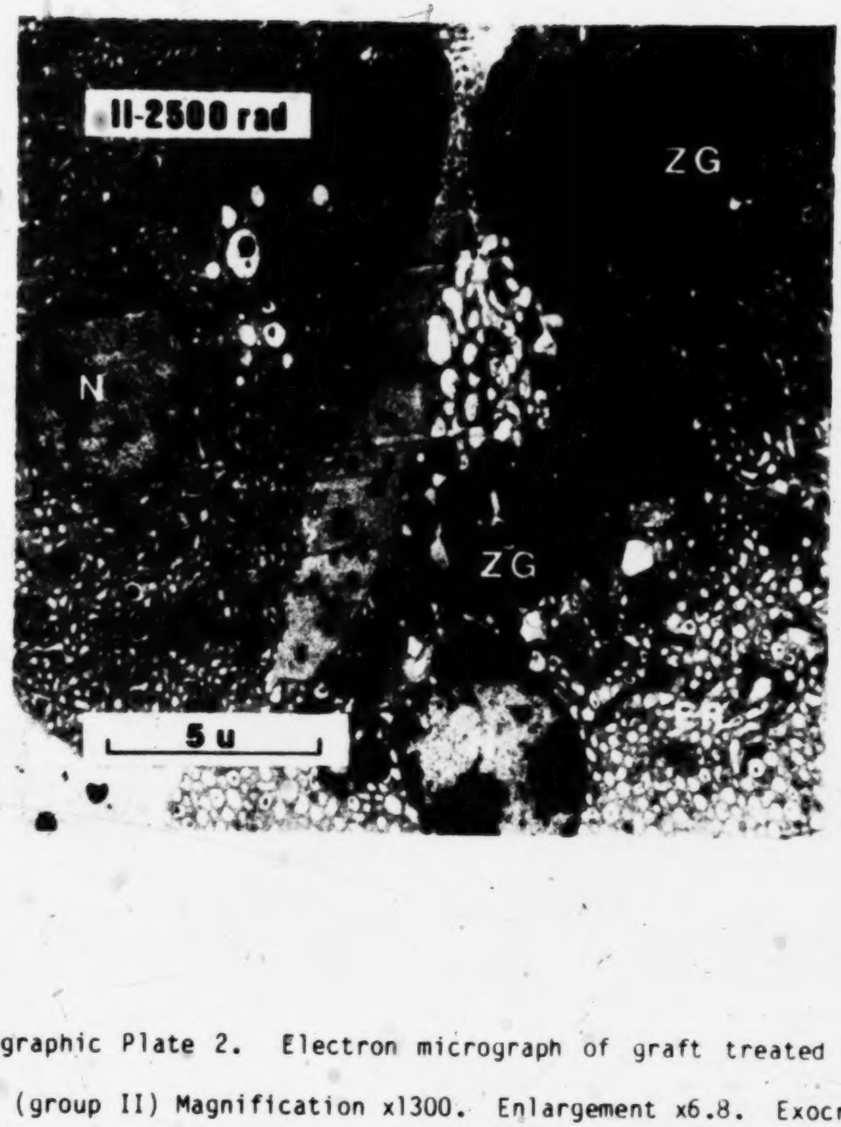
Exocrine tissue is seen in all 4 sections. Group I (Photographic Plate 1) and group II (Photographic Plate 2) appear similar. Normal exocrine tissue is seen. Dark staining zymogen granuals fill the cytoplasm. The nucleus appears normal. The endoplasmic reticulum is visible. In group III (Photographic Plate 3) the exocrine tissue appears altered. The nucleus appears shrunken and pyknotic. The cytoplasm is featureless. The endoplasmic reticulum is no longer visible. There is a general decrease in intracellular organelles. The number of zymogen granuals appears to be decreased. In group IV (Photographic Plate 4) the injury pattern is more marked. There is hydropic swelling in the region of the nucleus. Numerous vocuoles fill the cytoplasm. Mitochondria appear swollen. Cellular and nuclear membranes are no longer distinct.

Electron microscopy of the graft after 8h and 24h of tissue culture revealed autolytic tissue only. There was no difference in the 4 groups.

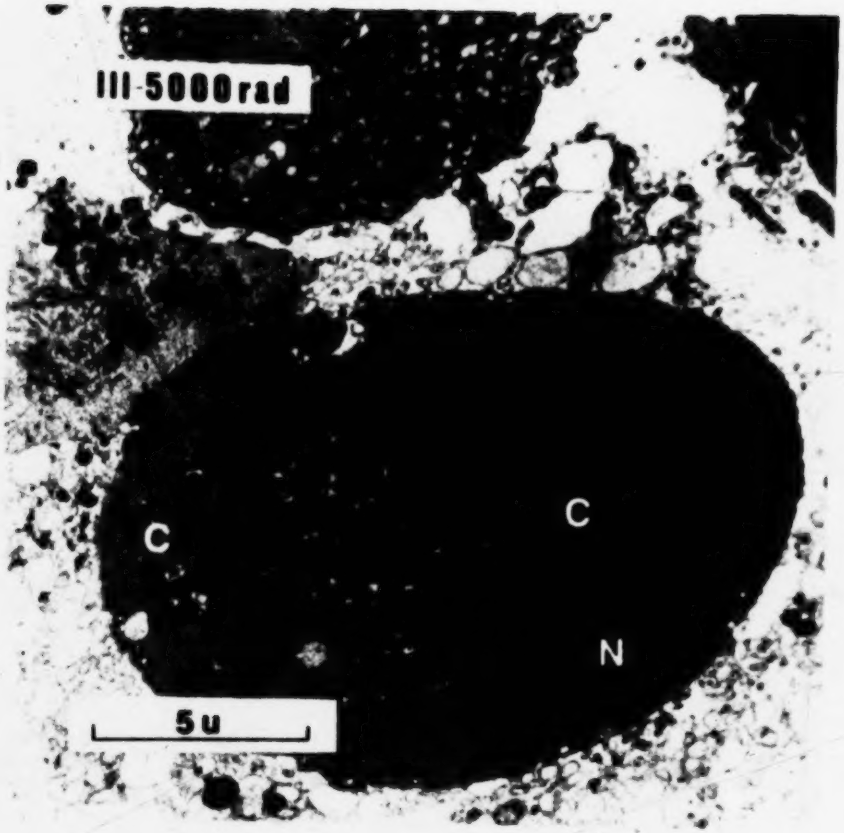
Islets were not identified in any of the sections reviewed.



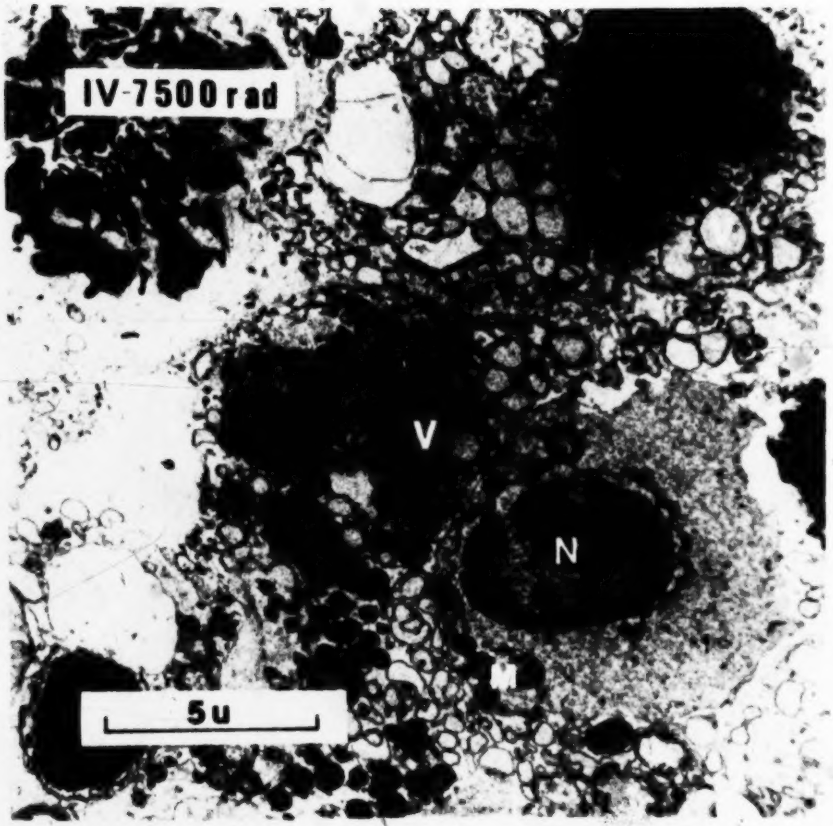
Photographic Plate 1. Electron micrograph of untreated graft (group 1). Magnification x1300. Enlargement x6.8. Exocrine cells are shown with nucleus (N), zymogen granuals (ZG) and endoplasmic reticulum (ER) labelled.



Photographic Plate 2. Electron micrograph of graft treated with 2500 rad. (group II) Magnification x1300. Enlargement x6.8. Exocrine cells are shown with nucleus (N), zymogen granuals (ZG) and endoplasmic reticulum (ER) labelled.



Photographic Plate 3. Electron micrograph of graft treated with 5000 rad (group III). Magnification x1300. Enlargement x6.8. Exocrine cells are shown with nucleus (N) and cytoplasm (C) labelled.



Photographic Plate 4. Electron micrograph of graft treated with 7500 rad (group IV). Magnification x1300. Enlargement x6.8. Exocrine cells are shown with nucleus (N), intracytoplasmic vocuoles (V), and mitochondria (M) labelled.

PERIFUSION

In 7 of the 8 dogs the nonirradiated and irradiated tissue responded to glucose challenge in a similar fashion with an increase in insulin secretion. In 1 dog (Δ) the irradiated tissue did not respond with an increase in insulin secretion.The perifusion results compiled from the 8 dogs for groups I-IV are shown in Figures 10-13. A similar response was seen in all 4 groups. An initial baseline of insulin secretion was seen followed by a small peak in insulin secretion with glucose stimulation at 60 min. During the period of stimulation from 60 to 120 min. the rate of insulin secretion increased. When glucose stimulation was stopped at 120 min. the rate of insulin secretion decreased. During the post-stimulation period from 120 min. to 180 min. the rate of insulin secretion did not return to baseline in all 4 groups. This appeared to be more marked with increasing doses of radiation. The biphasic pattern of insulin secretion seen in all 4 groups indicates viability of the endocrine component of the graft. There was no significant difference in the rate of insulin secretion between groups I-IV of any point of the perifusion.

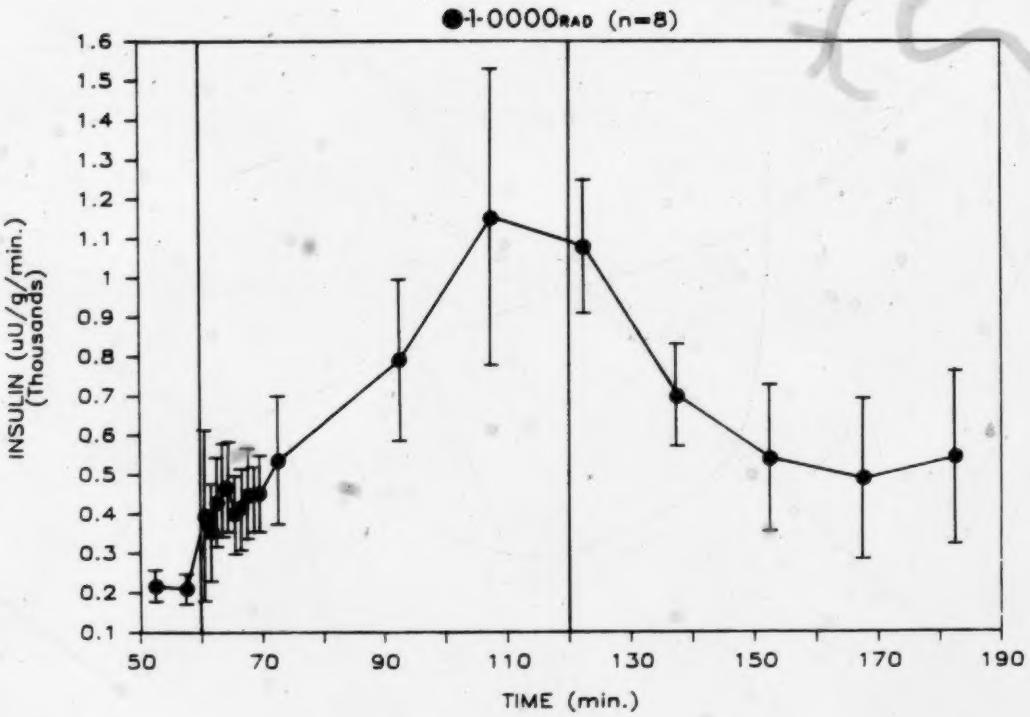


Figure 10. Pattern and rate of insulin secretion (mean ± SEM) from untreated graft (group I) with invitro glucose challenge in perifusion. Vertical lines at 60 min. and 120 min. indicate period of stimulation.

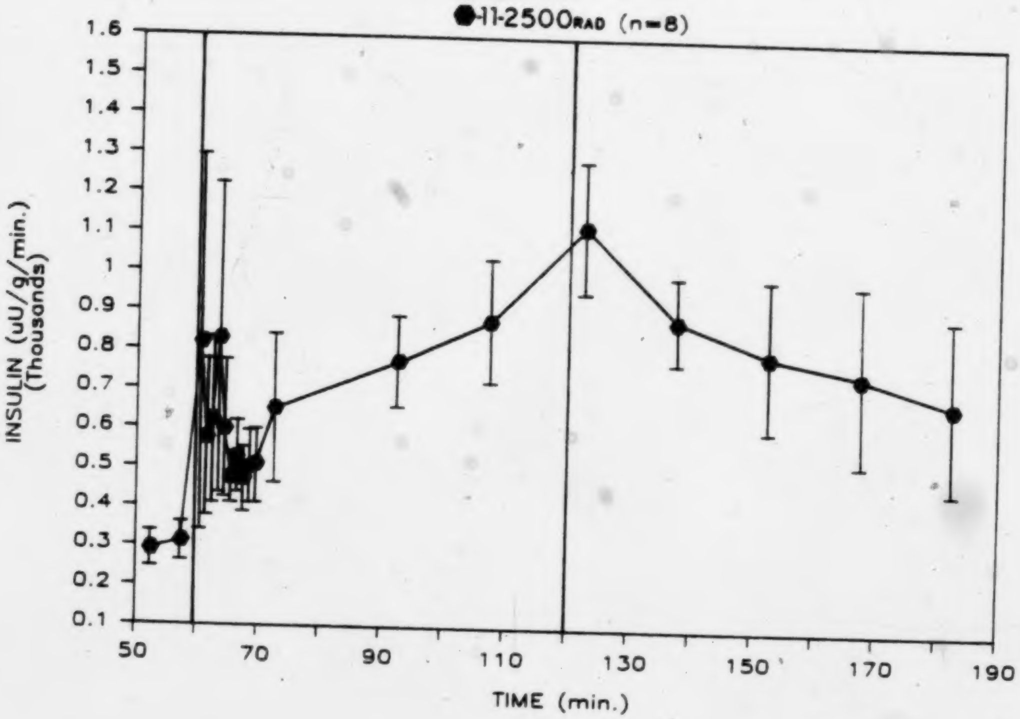


Figure 11. Pattern and rate of insulin secretion (mean ± SEM) from graft treated with 2500 rad (group II) with invitro glucose challenge in perifusion. Vertical lines at 60 min. and 120 min. indicate period of stimulation.

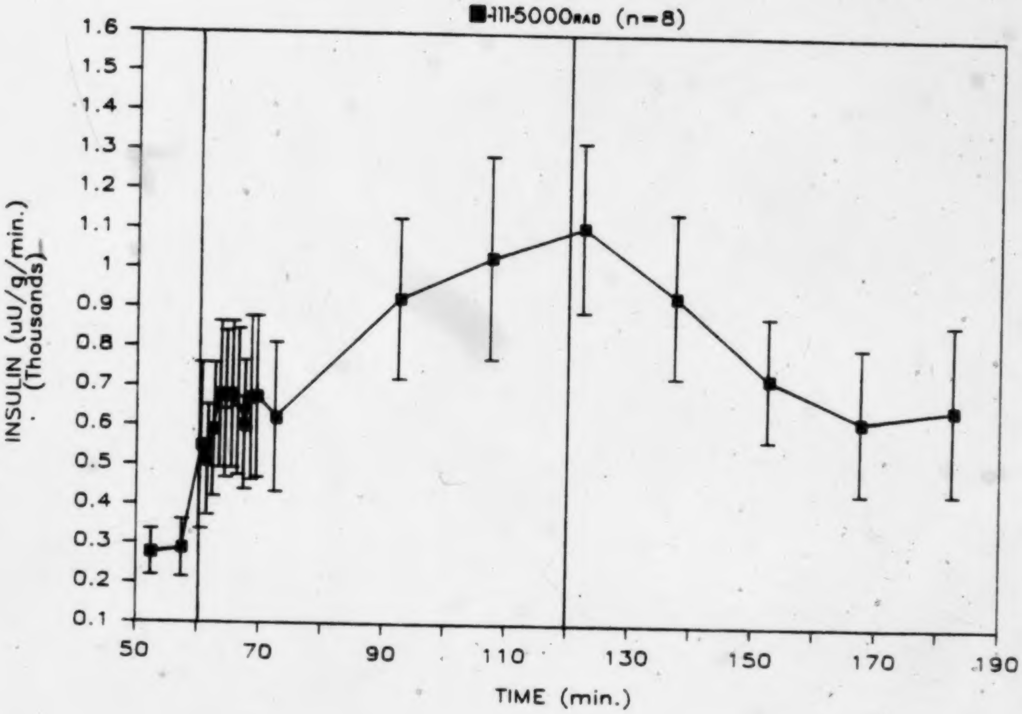


Figure 12. Pattern and rate of insulin secretion (mean ± SEM) from graft treated with 5000 rad (group III) with invitro glucose challenge in perifusion. Vertical lines at 60 min. and 120 min. indicate period of stimulation.

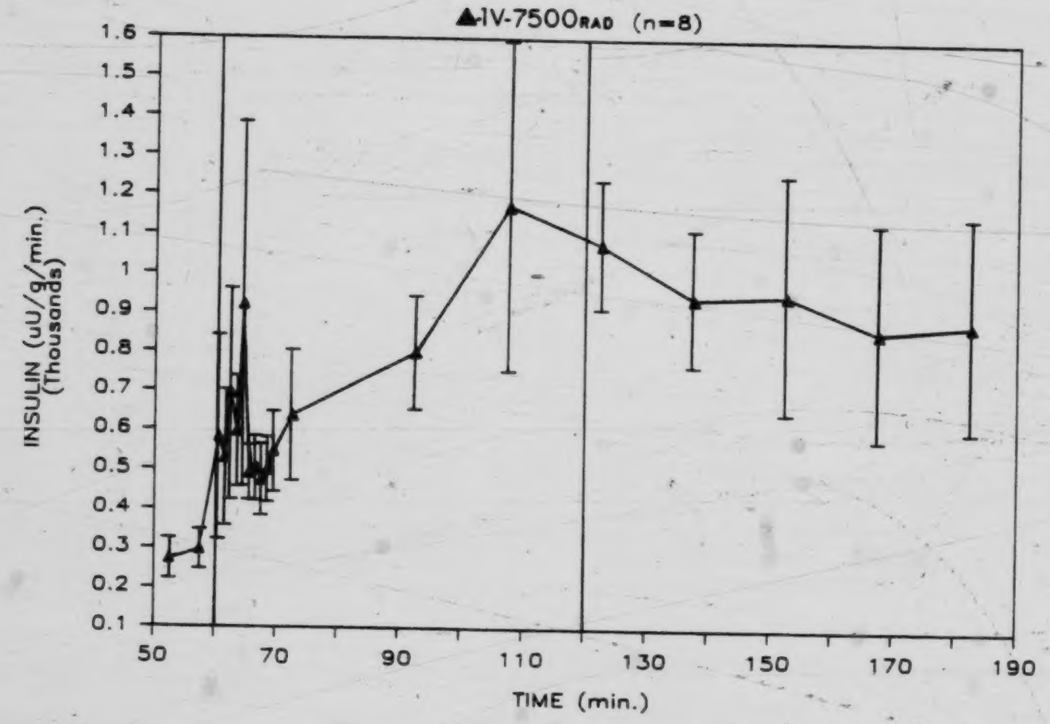


Figure 13. Pattern and rate of insulin secretion (mean ± SEM) from graft treated with 7500 rad (group IV) with invitro challenge with glucose. Vertical lines at 60 min. and 120 min. indicate period of stimulation.

INVIVO STUDIES

Apancreatic Controls

The 5 dogs became severely diabetic and survived for 10 ± 2 days. The mean blood glucose was 446 ± 33 terminally.

Intrasplenic Autografts

The assessment of islet recovery and purification in group Ia (nonirradiated autografts) and group IIa (irradiated autografts) is shown in Table 5.

Table 5. Insulin content and I/A ratio of pancreas and grafts in group Ia, nonirradiated autografts and group IIa, irradiated autografts. (mean ± SEM).

		Spun Volume (ml)	Insulin Content (uU/g) (thousands)	Approx. Total Insulin Content (uU) (thousands)	I/A ratio
Ia	Pancreas	58.9 ± 5.5*	3.55 ± 1.50	193.94 ± 68.90	17.0 ± 11.8
	Untreated graft	19.1 ± 1.7	2.62 ± 0.34	50.18 ± 6.96	4.7 ± 1.8
IIa	Pancreas	59.7 ± 3.7*	2.76 ± 0.80	159.25 ± 45.54	1.1 ± 0.4
	Untreated graft	17.6 ± 0.7	2.01 ± 0.19	36.16 ± 45.19	1.9 ± 0.5
	Treated graft	12.7 ± 0.7	2.14 ± 0.19	27.09 ± 2.74	1.8 ± 0.3

*weight (g)

Autograft survival at 1 month after transplant is summarized in Table 6. In group Ia there were two technical failures. In one dog the splenic hilar area was injured during removal of the cannulas. The other dog died on day 2 postimplant from gangrenous bowel secondary to a massive small bowel intussusception. All engraftments were technically successful in Group IIa. In Group Ia one dog died from graft failure on day 28 postimplant. In Group IIa, two dogs died from graft failure, both on day 26 postimplant. The survival of technically successful autografts at 1 month postimplant was, 6 of 7 dogs in group Ia and 5 of 7 dogs in group IIa.

Table 6. Autograft survival at 1 month after transplant (No. of dogs).

	Ia Nonirradiated autografts (n=9)	IIa Irradiated autografts (n=7)
Technically successful grafts	7	7
Death from graft failure before 1 mo	1	2
Survival at 1 mo.	6	5

In group Ia the 6 dogs surviving at 1 mo. were normoglycemic (fasting serum glucose < 150 mg/100ml). In group IIa, 4 of the 5 dogs surviving at 1 mo. were normoglycemic. The mean fasting serum glucose levels recorded during the first 4 week postimplant in the dogs which were normoglycemic at 1 mo. are shown in Figure 14. The differences observed in the means were not significant.

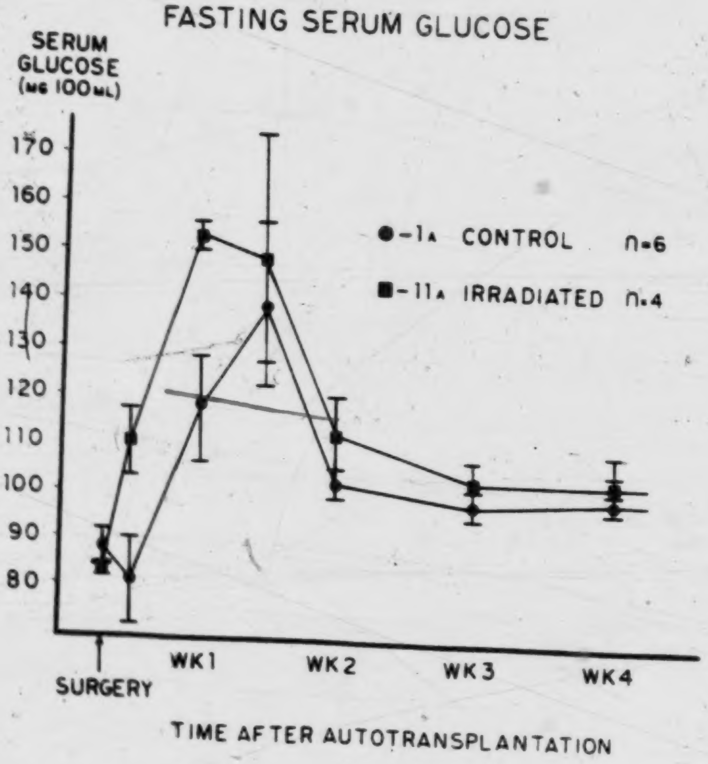


Figure 14. Fasting serum glucose concentrations (mean ± SEM) during the first 4 weeks after intrasplenic autotransplantation in dogs normoglycemic at 1 mo. postimplant.

Autograft function in dogs normoglycemic at 1 month postimplant is summarized in Table 7. There was no significant difference between the two groups at 1 mo. postimplant with respect to percent wt. loss. The K value and peak insulin response in group IIa were significantly lower ($p < .001$) than observed in group Ia.

Table 7. Autograft function at 1 month after transplant.

	Ia Nonirradiated Autografts (n=6)	IIa Irradiated Autografts (n=4)
Wt. loss at 1 month (percent of preop. body wt.)	8.0 ± 1.6	8.0 ± 2.1
K value during ivGTT (percent decline in glucose)	1.66 ± 0.25	0.93 ± 0.16
Insulin peak during IVGTT (uU/ml)	7.2 ± 0.9	2.0 ± 0.7

The serum glucose recorded during ivGTT in both groups, preoperatively, and at 1 mo. postimplant, is shown in Figure 15. The preoperative K values for group Ia and group IIa, 3.81 ± 0.37% and 3.74 ± 0.35% respectively, were similar. The decrease in the K values seen at 1 mo. postimplant from the preoperative levels was significant in group Ia ($P < 0.01$) and group IIa ($P < 0.05$). The mean K value for group IIa fell within the diabetic range ($K < 1.0\%$).

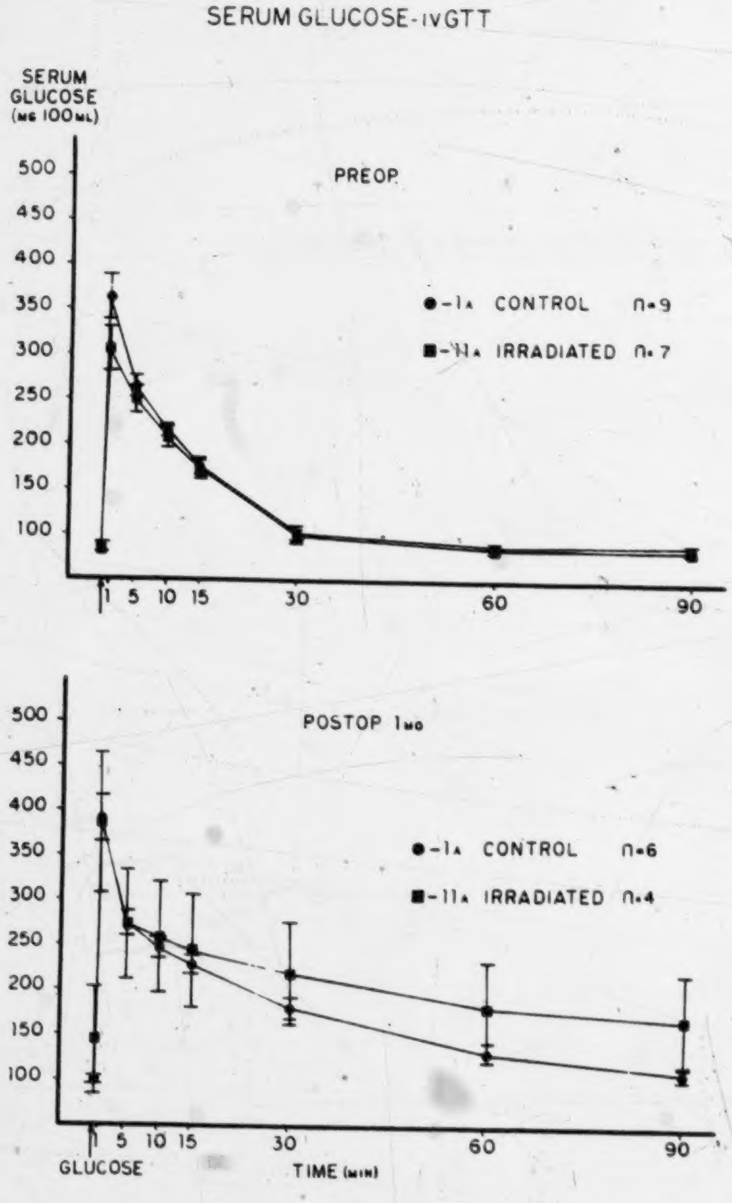


Figure 15. Serum glucose (mean ± SEM) recorded during ivGTT preoperatively (top) and at 1 mo. after transplantation (bottom).

The plasma insulin recorded during ivGTT in both groups, preoperatively, and at 1 mo. postimplant, is shown in Figure 16. The peak insulin response in group Ia, 54.9 ± 9.7 uU seen at 10 min. and in group IIa, 40.8 ± 13.0 uU seen at 15 min. was not signficantly different. The peak insulin response during ivGTT at 1 mo. postimplant in group Ia was significantly ($P < 0.05$) lower than the preoperative level. In group IIa the peak insulin response at 1 mo. was significantly ($P < 0.05$) reduced from the preoperative level as well, and appeared to be shifted to the left.

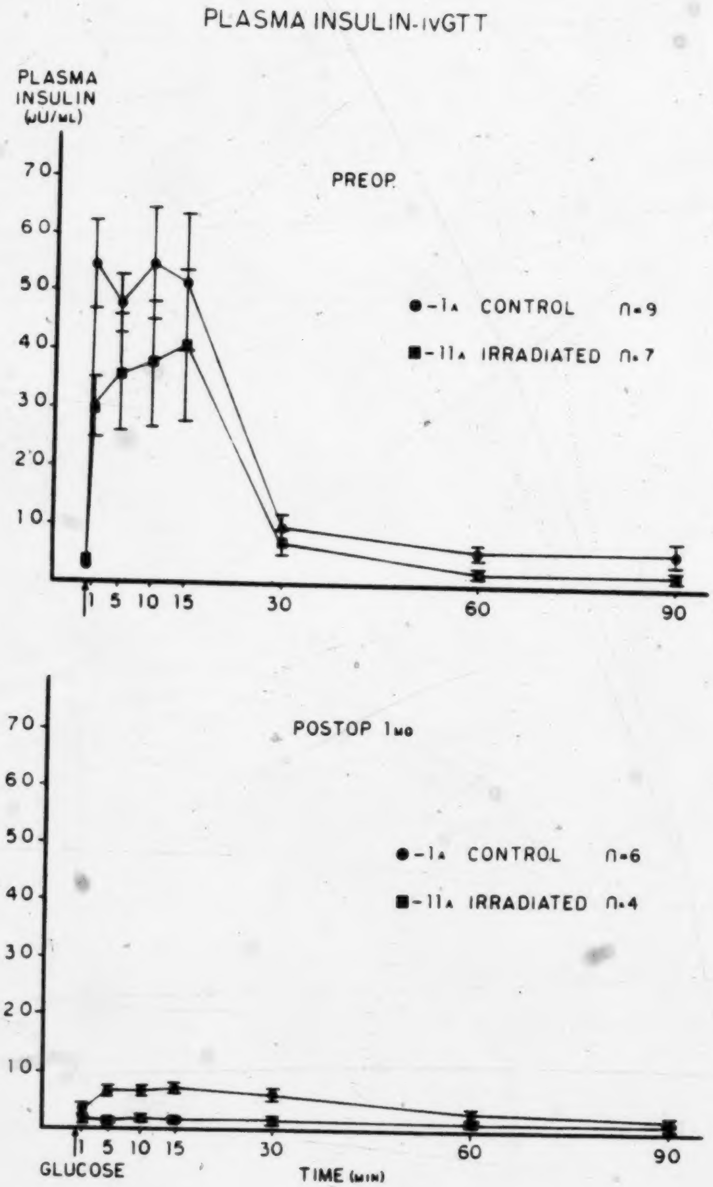


Figure 16. Plasma insulin (mean ± SEM) recorded during ivGTT preoperatively (top) and at 1 mo. after transplantation (bottom).

IX DISCUSSION

Experimentally pancreatic islet cell transplantation is very successful. In syngeneic strains of rodents isolated and purified islets from multiple donors infused into the portal vein of diabetic recipients can completely normalize serum glucose and insulin (9,49,78, 79,90) and reverse microvascular complications (18,19,41,52-54). The techniques used to isolate and purify islets in rodents cannot be adapted to the fibrous mammalian pancreas. (10,20,21,91-94) Clinical trials of pancreatic islet cell transplantation have not been successful. (11,69, 70) The major barrier to clinical success at the present time is the inability to harvest a sufficient volume of viable islets from a single pancreas to completely restore metabolic function when transplanted to a diabetic recipient. A partial solution to this problem in the canine model has been to eliminate the steps of purification used in the rodent and transplant pancreatic fragments (20,21) consisting of islet cells as well as contaminating exocrine, endothelial,ductal and immune cells. In the canine model pancreatic fragments from one donor pancreas transplanted into the spleen of an apancreatic recipient can maintain long-term normoglycemia (20-22,91,95-101) but metabolic function is not completely restored to normal (20-22,99-101).

The contaminating components of the graft, particularly the exocrine cells have been responsible for reports of severe complications including disseminated intravascular coagulation (102,103) and liver dysfunction (102, 129) following intraportal infusion of these grafts clinically and in experimental models. Contaminating components of the graft that express Class II antigens, the sole stimulators of certain immune responses (10, 28,117), have been implicated in the vulnerability of islet cell grafts to allograft rejection. Class II antigens are found in high concentrations on dendritic cells or fixed tissue macrophages and are present on ductal and vascular endothelial cells and exocrine cells. Islet cells appear to be devoid of Class II antigen. (10,117,120,121) In the rodent model, by treating isolated and purified islets to remove dendritic cell function or Class II antigen from the graft, allograft survival can be prolonged without systemic immunosuppression. (25,26,113-117,150-152) This is an extension of the "passenger leukocyte" concept initially prepared by Snell in 1957.

A pure preparation of islets is therefore desirable for immunologic reasons as well as the safety of the transplant procedure. For these reasons considerable effort has been directed at purifying preparations of pancreatic fragments. The majority of methods involve tissue culture techniques. At least a 24 h period of tissue culture in combination with antidendritic cell antibody or ultraviolet irradiation, for example, is necessary to remove dendritic cell function from a graft. The preparation of relatively pure suspensions of "pseudoislets" by elutriation and gyrorotational culture of single cell preparations of islets is being investigated. (10,29,130,148) A major problem with the above techniques as well as other methods being investigated to purify pancreatic fragments is the loss of insulin secreting tissue during the purification process. (19,26,29,141,142,143) As a consequence of this low islet recovery after purification attempts to autotransplant islet cell grafts purified by the above means has not been successful in large animal models. (10,130,141,142,153) In fact it has not been possible to test the theory of immunoalteration in the canine model.

For clinical islet cell transplantation to be successful graft preparation has to be improved to increase islet yield and more efficient means to purify graft preparations are necessary. In general, a practical, clinically applicable, and efficient means to separate the endocrine component of the pancreas from contaminating exocrine cells and other components is necessary. In this study experience with high dose radiation as a new means to purify a graft preparation of pancreatic fragments in the canine model has been documented.

EFFECTS OF HIGH DOSE RADIATION ON THE CANINE PANCREAS

The findings in this study support and are consistent with evidence from previous investigations (170-188) that radiation in high doses can injure the exocrine component of the canine pancreas and leave the endocrine component viable. The changes in acinar cells that have been previously documented include: degranulation (173,180,186), intracytoplasmic inclusion bodies (173,186), nuclear changes (180), necrosis and atrophy (171,172,177,180). Parallel with these structural changes a decrease in enzyme content of the pancreas (173) and secretion of enzymes (175,177,178,179) have been documented. The changes observed progress with time. (173,175,176) Structural changes in beta cells, when observed, have been minimal (173,177,180) consisting predominantly of degranulation. (173) Dogs remain normoglycemic following high doses of radiation to the pancreas. (173,175-177)

In contrasting the findings in this study to those in previous investigations a consideration of the experimental models used is necessary. All previous studies in the canine model have involved radiation of the intact and vascularized gland. The general approach has been to exteriorize the pancreas and radiate the gland followed by creation of a controlled pancreatic fistula for functional studies (174,175,177) or sacrificing the animals at various intervals after radiation to sample the pancreas for structural changes. (171-177,180) An example of this type of experimental model is that used in the studies of Volk and associates. (173,176) They delivered doses varying from 5000 to 9000 rads, single fraction, to the exteriorized canine pancreas. Dogs were sacrificed at intervals from 30 min to 21 days after radiation and the pancreas sampled and examined for enzyme content and structural changes.

There are several problems with the above models that are illustrated in the example given. The first is that the changes observed, particularly toward the end of the observation period, could be secondary to radiation induced vascular changes in the gland as opposed to primary radiation injury to the components of the pancreas. Casarett (168) and Archembeau (174) feel that the differential susceptibility of the exocrine and endocrine pancreas to high dose radiation can be explained on the basis of vascular injury. Another problem is the accuracy of the dose of radiation. In the example given, the study of Volk and coworkers, changes in the pancreas at 21 days were contrasted to changes in the pancreas at 30 min following radiation. Comments were made on the progression of radiation injury in the pancreas with time. The results are difficult to assess as one dog may have received 5000 rad and the other 9000 rad. In the example cited as well as other studies reviewed no attempt was made to establish a dose-response relationship. Inaccurate doses of radiation would make dose-response relationships difficult to assess even if such considerations were incorporated into the experimental design. In making comparisons between the previous studies and the present investigation, an important factor is that the dose, dosage schedule, rate of delivery, and type of radiation was different in each study. For example Zook and coworkers (180) delivered doses up to 6750 rad of photons in 4 fractions per week for 6 weeks. Rauch and Stenstrom (175) used a 250 KV X-ray machine to deliver doses up to 1000 rad over a 6 week period. The study of Volk and coworkers is the only investigation in which the dose of radiation used (5000 to 9000 rad, single fraction) is somewhat comparable to the doses used in the present study.

The experimental model used in the present study has several unique features. The first is that a suspension of pancreatic cells was irradiated and not the intact vascularized gland. As a consequence changes secondary to radiation induced vascular injury are not a consideration. The other feature is that three separate doses of radiation were delivered very accurately to the pancreatic cells of one pancreas and the results compared to a control from the same pancreas. It was therefore possible to compare and contrast the effects of several different doses of radiation on the pancreas and make inferences concerning dose-response relationships. To obtain a similar volume of data using the intact vascularized pancreas four times the number of dogs would have to be used. As well, another consideration would become necessary, the variability between individual dogs. This variability was marked and statistically significant in this study. The variability could be accounted for by such factors as differences in age and inherent differences in radioresponsiveness between the dogs.(165) Another factor, and a disadvantage of the model used in the present study, is the imprecise nature of the graft preparation, particularly the collagenase digestion step (10,29,92,93) resulting in different relative quantities of viable endocrine tissue as well as contaminating exocrine tissue in each graft.

However, the experimental design used in this study permitted the variability between dogs to be eliminated in the statistical analysis of the data.

In the present investigation the structural changes consistently seen in the acinar tissue after high doses of radiation included degranulation, intracytoplasmic vacuoles and nuclear changes. Changes in mitochondria and endoplasmic reticulum were observed as well. These changes were not apparent after a dose of 2500 rad and appeared to be progressive with the higher doses of radiation. Intracytoplasmic inclusion bodies as described by Volk and associates (173,176) were not seen. The authors felt these inclusion bodies represented autophagic lysosomes. According to Swift (197) autophagic lysosomes indicate a reparative process in the cell and represent a non-specific response to injury. Intracytoplasmic vacuoles, nuclear alterations, and changes in the mitochondria and endoplasmic reticulum are patterns of injury described in irradiated cells. (159)

In this study the amylase content of the pancreas did not consistently decrease with increasing doses of radiation. The mean response demonstrated a very marginal and insignificant decrease in the irradiated group compared to the control. In the study of Volk and coworkers (173) the amylase content of the irradiated pancreas was less than the amylase content of the control dogs. This decrease was most apparent in dogs sacrificed 5 to 8 days after radiation. The results reported in this study were from tissue sampled immediately after radiation. The amylase activity measured in the supernatant of the graft immediately following radiation consistently decreased with increasing doses of radiation. This decrease in amylase activity could be a result of a radiation induced decrease in protein synthesis (163) or a decrease in enzymatic activity secondary to structural alterations of the enzyme from radiation induced changes in chemical bonds. (163,164) The functional response of the exocrine pancreas to high dose radiation was not assessed in this study.

In the present investigation endocrine cells were viable immediately after radiation with doses up to 7500 rad. This was indicated by a biphasic pattern of insulin release with glucose stimulation in perifusion. After stimulation the rate of insulin secretion did not return to the baseline level in the control and the irradiated groups. This feature appeared to become more pronounced with increasing doses of radiation. This could indicate a degree of injury to the beta cells. A similar pattern, failure to return to baseline levels of insulin secretion poststimulation, is seen with perifusion of cryopreserved canine pancreatic fragments in this lab. The viability of these cells has been established by their ability to maintain long-term normoglycemia in apancreatic dogs following intrasplenic autotransplantation. (126) In this study metabolic function at 1 mo. postimplant was significantly better in the control series of dogs than in dogs receiving a graft treated with 5000 rad. This again might indicate a degree of injury to the beta cells following a dose of 5000 rad, single fraction. However, the irradiated graft was transplanted approximately 1.5 h after graft preparation. In the control series the untreated graft was transplanted 15 to 20 min. after graft preparation. The possibility that islet viability could be affected by the manipulations involved in the radiation of the graft as opposed to radiation injury per se has to be considered. In retrospect, the graft used in the control animals should have been taken through the same manipulations as the irradiated graft including suspension in tissue culture media, transport to and from the Cross Cancer Institute and resuspension in chilled Hank's solution. In the studies of Volk and associates (173,176) dogs remained normoglycemic and had normal glucose tolerance tests during a 12 mo. period of observation following doses of radiation similar to that used in this study. Blood glucose has remained normal following radiation of the pancreas in other studies (175,177-179), however the dose of radiation was generally less than that administered in the present investigation. In my opinion, the viability of endocrine cells one month following a dose of 5000 rad, single fraction, remains in question. The biological effects of radiation may not become manifest for a period of time after initial injury. (159)

It would be interesting, using the same experimental model, to see if similar changes in the acinar tissue could be induced with lower doses of radiation by modifying the conditions of radiation. Consideration could be given to one or more of the following. The pancreatic fragments could be irradiated at room temperature instead of 4°C. The radiosensitivity of cells can be enhanced by an increase in temperature. (159,162,166) Warm ischemia alone has been used to selectively destroy exocrine cells. (136) An hypoxic sensitizer such as metranidazole (166) could be added to the ductal perfusate prior graft preparation, the enzyme stop-solution during graft preparation or to the tissue culture media prior to radiation. The ability of oxygen to enhance the effect of radiation (oxygen effect) has been observed in invitro systems. (166) The dose could be fractionated, 2500 rad to the pancreas prior graft preparation and 2500 rad after graft preparation for example. There is evidence that fractionated doses of radiation accelerate repair of cells and that some acquired resistance or adaptation may occur within viable cells. (159,162) Particulate radiation with a higher LET could be used. The ideal objective would be to achieve interphase necrosis (162) of exocrine and other contaminating cells, leaving endocrine cells viable.

The findings in this investigation that radiation in high doses can injure exocrine cells and leave endocrine cells viable, at least immediately after radiation, are consistent with previous reports of the effects of high dose radiation on the intact and vascularized pancreas. It cannot be concluded that the injury is entirely selective as there may be a degree of injury to the beta cells. Unlike previous investigations the changes observed in the pancreas in this study cannot be attributed to radiation induced vascular injury.

The findings in this study as well as in previous investigations are not entirely consistent with the traditional concepts of radiosensitivity as originally proposed by Bergonie and Tribondeau. (162,163) Their theory has generally been interpreted to mean that actively proliferating cells are the most sensitive to radiation and that the radiosensitivity of cells varies inversely with their degree of differentiation. The implication of the concept is that cells which do not divide are radioresistent. The term "radiosensitivity" as it is currently used referes to the sensitivity of the process of cell division rather than the functional integrity of the cells in the irradiated tissue. Both endocrine and exocrine cells represent a fixed post-mitotic population of cells. (168) The differential susceptibility of the components of the pancreas to high dose radiation is consistent with the more recent concepts of radioresponsiveness. It has been proposed that all cells are radiosensitive if a dose of sufficient strength is administered and different cell populations vary in the dose required to injure the cell irrespective of their reproductive capacities. (159,161,162,168)

Detailed studies of the histopathological and functional response of the pancreas to high dose radiation have not been done. The model used in the invitro aspect of this study, with the addition of functional assessment of exocrine cells by invitro challenge with secretagogues in perifusion,would provide a good basis for a detailed examination of the immediate effects of radiation on the components of the pancreas.

HIGH DOSE RADIATION AS A MEANS OF GRAFT PURIFICATION

Under the conditions defined in the invitro aspect of this study radiation in doses of 5000 to 7500 rad, single fraction, delivered to a graft preparation of pancreatic fragments can significantly purify the untreated graft by a factor of 2 to 3. Treatment of the graft with 5000 rad significantly increased the insulin:amylase ratio 7 to 8 fold over whole pancreas. Graft preparation alone insignificantly increased the insulin:amylase ratio 2 to 3 fold over whole pancreas.

The increase in the insulin concentration and the insulin:amylase ratio of the graft was more pronounced than expected from the insignificant decrease in the amylase concentration. This suggests that a decrease in contaminating components of the graft, other than exocrine tissue,is responsible in part for the purification seen. Contaminating components other than exocrine tissue would include ductal, endothelial, connective tissue, and specific immune cells. As well, the degree of purification seen cannot be attributed entirely to radiation. Although the mean respone was not significantly different from the untreated graft or whole pancreas, the control group consistently had a higher insulin:amylase ratio than the untreated graft. A part of the purification seen could therefore be attributed to the manipulations involved in the radiation. The pancreatic fragments were suspended in chilled Hank's balanced salt solution for approximately one hour, the time spent in transporting and irradiating the tissue. It would appear that a short period of cold ischemia alone can increase the insulin:amylase ratio.

The assessment of islet recovery and purification in the invivo studies was not consistent with the results obtained in the invitro studies. The mean insulin:amylase ratio of the irradiated graft was lower than that of the untreated graft. More surprising than this was the magnitude of the mean insulin:amylase ratio of the pancreas. In the control group the ratio was 3 to 4 fold higher than that of the untreated graft, and approximately twenty-five times greater than the mean insulin:amylase ratio of the eight pancreases used in the invitro studies. There is one consideration that could explain a part of the observed discrepancy. In the invitro studies the tissue was weighed and frozen for subsequent insulin and amylase assay immediately after sampling. In the invivo studies the tissue was placed in a refrigerator until completion of the transplant procedure at which time the samples were weighed and then placed in a freezer. In other words these samples were subjected to a period of cold ischemia which was approximately 3 to 4 h for the pancreas, 2 to 3 h for the untreated graft and 1 to 2 h for the irradiated graft. As demonstrated in the invitro studies the insulin:amylase ratio can increase with as little as 1.5 h of cold ischemia. In retrospect the tissue should have been frozen immediately after sampling in the invivo studies. From the above consideration it is my opinion that the assessment of islet recovery and purification in the invivo studies is inaccurate and should be excluded from evaluation in this study.

In a series of studies reported by Matas and associates (141,142), tissue culture for a period of 24 h increased the insulin:amylase ratio of pancreatic fragments prepared from the canine pancreas by a factor of six over whole pancreas. The purification was associated with a rapid and significant decrease in the amylase concentration of the graft. As well the insulin concentration decreased to less than fifty percent of its original level during the first 4 h of tissue culture and then stabilized. These results are similar to the findings from the tissue culture studies in this investigation. For example, the insulin:amylase ratio of the nonirradiated graft was purified by a factor of 11 over whole pancreas in association with a significant decrease in amylase concentration and a greater than fifty percent reduction in insulin concentration during 24 h of tissue culture. The greater degree of purification achieved and the maintenance of the insulin concentration of the graft for the first 8 h of tissue culture in this study is probably a result of the volume of tissue culture media used. Matas and associates used 25 ml per g of graft while in this investigation 150 ml per g of graft was used.

The observation that an accelerated destruction of exocrine tissue relative to islet tissue occurs in tissue culture was first observed by Lazarow and associates. (140) One explantation for this phenomenon is that exocrine cells are autodigested rapidly due to the action of intrinsic digestive enzymes. Islet cells intrinsically lack the potent digestive enzymes and although some islet loss is inevitable from the action of released enzymes they are relatively spared. The addition of an enzyme inhibitor such as Trasylol to the culture media has been shown to significantly improve islet recovery in tissue culture by protecting the islets from the released enzymes. (141,142)

The trends seen in the tissue culture were similar for both the nonirradiated and irradiated tissue. The observation that the untreated graft appeared to benefit more in terms of purification than the irradiated graft is not surprising as the irradiated graft was partly purified when placed in tissue culture. The only significant difference in the performance of the irradiated and nonirradiated graft was the decrease in insulin from 8 h to 24 h of culture observed in the graft treated with 7500 rad. This could reflect a loss of islet viability secondary to radiation injury after this dose or possibly increased damage by released exocrine enzymes secondary to accelerated destruction of exocrine cells. The decrease in amylase concentration from 8 h to 24 h of culture appeared to be faster in the groups receiving the higher doses of radiation.

Islet cell grafts purified by tissue culture (141,142) and relatively pure preparations of aggregated islets or "pseudoislets" (10,130) have been autotransplanted in the canine model. Matas and coworkers (141,142) transplanted pancreatic fragments cultured for 24 h to 15 diabetic dogs. Six dogs were reported surviving and normoglycemic at 1 mo post-implant. There are two important considerations in evaluating this study. The model for diabetes was partial pancreatectomy and streptozotocinin. One of 14 diabetic control dogs was normoglycemic at 1 mo. Transplantation involved infusion of fifty percent of the graft intraperitoneally and fifty percent intraportally. It is therefore difficult to compare the results of this study to the present investigation or other recent studies where pancreatic fragments are autotransplanted to the spleen in totally pancreatectomized dogs. Scharp and associates (10,130) report that 25 percent of apancreatic dogs receiving an intrasplenic autograft of pseudoislets are normoglycemic at 1 mo post-implant. Lorenz and coworkers (104) have reported successful transplantation studies using Ficoll separated islets in the canine model. This work has not been reproducible in other laboratories. It is generally felt that density gradient sedimentation, used successfully to purify islets in rodents, cannot be applied to the fibrous mammalian pancreas. (10,20,31,91-94) The newer methods of purifying islet cell grafts being investigated, for example counter-current electrophoreisis in zero gravity have not reached a level of practicality or efficiency that will permit transplantation studies.

The use of radiation to purify pancreatic fragments has not been previously reported. In this study survival of apancreatic dogs receiving an intrasplenic autograft of pancreatic fragments treated with 5000 rad was considerably better than apancreatic controls. Although metabolic function was significantly poorer than that observed in dogs receiving untreated grafts, the 70 percent survival at 1 mo post-implant suggests that the recovery of viable islets after this means of purification is better than with previously reported methods of purifying islet cell grafts in the canine model.

In summary, assuming the insulin:amylase ratio is an index of graft purity, radiation in high doses can purify graft preparations of pancreatic fragments in the canine model. The increase in the insulin:amylase ratio approximates that observed with 24 h of tissue culture. There are two important differences in grafts purified by 24 h of tissue culture and grafts purified by radiation. Although radiation is is associated with injury to exocrine cells there is not a significant decrease in the amylase concentration of the purified graft as seen with tissue culture. In contrast to grafts purified by tissue culture there appears to be an increase in insulin concentration of the graft purified with radiation. The recovery of viable islets following high dose radiation appears to be superior than that seen following 24 h of tissue culture.

APPLICATION OF HIGH DOSE RADIATION TO PANCREATIC TRANSPLANTATION

The ultimate barrier to clinical success of immediately vascularized pancreas and islet cell grafts is allograft rejection. More immediate problems of a technical nature face both forms of transplantation. The technical problem with immediately vascularized pancreas grafts is controlling the exocrine secretion of the pancreas. For islet cell transplantation the technical problem is islet yield.

With respect to allograft rejection one of the major advantages of islet cell transplantation over immediately vascularized pancreatic grafts is the potential to reduce immunogenecity of the graft invitro prior transplantation. This potential has been convincingly demonstrated in the rodent model (25,26,113-117,150-152) and in principle applies to the canine model. (26) The loss of insulin secreting tissue or low islet recovery associated with techniques used to purify grafts has prevented use of this theory to demonstrate prolonged allograft survival without immunosuppression in large animal models. (26,153) The reason the work has been successful in rodents is that islets can be pooled from multiple syngeneic donors.

It cannot be concluded that dendritic cell function was removed or even decreased in the purified graft preparation obtained in this study. However, it is very likely that the antigenecity of this graft preparation is reduced. Smaller doses of radiation than used in this investigation can destroy lymphocytes and macrophages. (165) The "passenger leukocyte" concept as it applies to pancreatic islet cell transplantation involves removal of other contaminating components of the graft expressing Class II antigen . A pure preparation of islets is necessary. Radiation in doses of 5000 to 7500 rad achieved significant purification of the graft in this study. Two previous studies demonstrate that pretreatment of pancreatic tissue with radiation can reduce antigenecity of the graft and that doses of the magnitude used in this investigation may be necessary to achieve this without the use of tissue culture. Lacy and coworkers (189) reported that pretreatment of donor rats with a total body dose of 850 rad prolonged survival of Ficoll separated and hand-picked islets across a minor histocompatibility barrier provided the islets were cultured for 24 h. Tersigni and coworkers (186) demonstrated prolonged allograft survival of immediately vascularized segmental grafts treated invitro with high doses or radiation. Animals receiving grafts treated with 5000 rads survived on average of 27.5 days and treated with 10,000 rad on average of 39.5 days. Treatment of the graft with 1000 rad resulted in an average allograft survival of 19 days.

The survival of nonimmunosuppressed islet cell allografts, using the same techniques for graft preparation and transplantation as described in this study, have been previously reported from this lab. (198) The mean time interval to the appearance of hyperglycemia and the mean survival was 5.0± 0.6 days and 16.0± 3.4 days respectively. Islet recovery following purification of the graft with radiation, as reflected by the 70 percent autograft survival of 1 mo post-implant, would be adequate to test the theory of immunoalteration in the canine model.

Islet yield is the major barrier to clinical islet cell transplantation. This is reflected by the metabolic function of the control series of autografts at 1 mo post-implant in this study. The rate of decline of glucose during IVGTT was 50 percent of that observed preoperatively and the peak insulin during IVGTT was less than 25 percent of the preoperative level. These results are similar to the findings of other investigators in this lab (22) and in other laboratories. (21,96) It is not surprising that autotransplants with grafts purified by methods which are associated with a loss of insulin secreting tissue fail. That an increase in islet yield will solve the problem of completely normalizing metabolic function has not been demonstrated in the canine model for obvious reasons. The earliest experimental studies in rodents, using multiple syngeneic donors, established that transplantation of a larger number of islets improved metabolic function. (9,24,78,79)

Previous experimental work on rodents and the canine model has shown that pretreatment of donors or the pancreas with agents that selectively destroy or degranulate exocrine cells, such as DL-ethionine or pilocarpine, results in at least a two-fold increase in islet yield. (29,138,139) In this study radiation in high doses was shown to injure and degranulate exocrine cells. The pattern of injury seen with DL-ethionine is similar to that seen with radiation. (173) It would be interesting to irradiate the intact gland, invitro, prior graft preparation and assess islet yield. An increase in islet yield would theoretically permit any further manipulations of the graft necessary for purposes of immunoalteration, such as a short period of tissue culture, without precluding successful transplantation studies.

In diabetic rodents less islets are required to completely normalize metabolic function when infused into the liver as opposed to other transplantation sites. (9,80,83) The contaminating components of grafts of pancreatic fragments has precluded the safe and successful utilization of the liver as a transplantation site in large mammals. (102,103,129) The purified graft preparation obtained in this study could be used to examine this aspect of islet cell transplantation in the canine model.

High dose radiation has been applied to the technical problem of controlling exocrine secretion in immediately vascularized pancreas grafts. (183-186,188) The results have been variable. All attempts to suppress exocrine secretion of these grafts, the most successful being occlusion of the duct with synthetic polymer, are complicated by progressive fibrosis of the graft with an accompanying decrease in endocrine function. (12,13,62-68) A late consequence of radiation injury in intact vascularized organs is ischemia and fibrosis (159,163) and this has been demonstrated following radiation of the pancreas (171-176).This feature would severely limit if not be a contraindication to the invitro manipulation of immediately vascularized pancreas grafts with high dose radiation.

There is one final consideration in the potential application of high dose radiation to pancreatic transplantation. Radiation can induce neoplasia. Islet cell tumors have been reported to develop in mice following doses of 450 rad. (170) For this reason alone long-term follow-up of animals receiving grafts of irradiated tissue, in terms of years, is necessary before any consideration is given to clinical application of this tool to pancreatic transplantation.

X CONCLUSIONS

Radiation in high doses is a relatively simple, efficient, and practical means of purifying a graft preparation of pancreatic microfragments.

The increase in the insulin:amylase ratio in the graft immediately after high doses of radiation approximates that seen following 24 h of tissue culture. Unlike tissue culture the purification seen with radiation is not associated with a significant decrease in amylase concentration of the graft. This implies that a part of the purification is a result of destruction of other contaminating components of the graft. The purification with radiation, in contrast to 24 h of tissue culture, is not associated with a decrease in insulin concentration of the graft.

The recovery of viable islets in a graft treated with 5000 rad is sufficient to permit better autograft survival than previously reported following other means of graft purification in the canine model. A graft preparation, purified with radiation under the conditions defined in this study, could be used to test the theory of immunoalteration in the canine model.

Radiation in high doses can injure exocrine cells. From the results of this study it cannot be concluded that the injury is entirely selective as it is possible the beta cells are injured to a degree as well. The pattern of injury in exocrine cells is similar to that seen with chemical agents such as DL-ethionine. Selective injury to exocrine cells in the intact pancreas has been shown to enhance the yield of islets with graft preparation. Potentially radiation, in the doses used in this study or in smaller doses with modifications of the conditions of radiation to achieve equivalent effects on the exocrine tissue, could be used to increase islet yield.

The use of high dose radiation for the purpose of immunoalteration or to suppress exocrine secretion in immediately vascularized pancreas grafts is limited, if not contraindicated, because of radiation induced fibrosis of the gland.

Further experimental evaluation of the applications of high dose radiation to the field of pancreatic islet cell transplantation is justified.

XI BIBLIOGRAPHY

1. Cahill GF. Diabetes mellitus. In: Wyngaarden JB, Smith LH, eds. Cecil: Textbook of Medicine. Philadelphia: WB Saunders, 1982: 1052-1072.

2. Rossini AA. Why control blood glucose levels. Arch Surg; 1976: 3: 229-233.

3. Tchobroutsky G. Relation of diabetic control to development of microvascular complications. Diabetologia, 1978; 15: 143-152.

4. Tchobroutsky G. Metabolic control and diabetic complications. In: Brownlee M, ed. Handbook of Diabetes Mellitus, Vol. 5: Current and Future Therapies. New York: Garland STPM Press, 1981: 3-40.

5. Raskin P. Treatment of insulin - dependent diabetes mellitus with portable insulin infusion devices. Med Clin North Am, 1982; 66(6): 1269-1282.

6. Tamborlane WV, Press MC. Insulin infusion pump treatment of type I diabetes. Pediatr Clin North Am, 1984; 31(3): 721-734.

7. Unger RH. Benefits and risks of meticulous control of diabetes. Med Clin North Am, 1982; 66(6): 1317-1324.

8. Unger RH. Insulin-glucagon-somatostatin interactions. In: Rifkin H, Raskin P, eds. Diabetes Mellitus, Vol. 5. Maryland: Prentice Hall, 1981: 43-54.

9. Sutherland DER. Pancreas and pancreatic islet cell transplantation. I. Experimental Studies. Diabetologia, 1981; 20: 161-183.

10. Scharp DW. Clinical feasibility of islet transplantation. Transplant Proc, 1984; 16(3): 820-825.

11. Sutherland DER, Goetz FC, Chinn PL, Elick BA, Najarian JS. Pancreas transplantation. Pediatr Clin North Am, 1984; 31(3): 725-750.

12. Sutherland DER. Selected issues of importance in clinical pancreas transplantation. Transplant Proc, 1984; 16(3): 661-670.

13. Groth CG. Clinical pancreatic transplantation. Transplant Proc, 1984; 17(11): 302-306.

14. Lacy PE. Pancreatic transplantation as a means of insulin delivery. Diabetes Care, 1982; 5(suppl1): 93-97.

15. Barker CF, Naji A, Perloff LJ, Dafoe DC, Bartlett, S. Invited commentary. An overview of pancreas transplantation-biological aspects. Surgery, 1982; 92(2): 133-137.

16. Lacy PE, Kostianowsky M. Method for isloation of intact islets of langerhans from the rat pancreas. Diabetes, 1967; 16(1): 35-39.

17. Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. Surgery, 1972; 72(2): 175-186.

18. Mauer MS, Sutherland DER, Steffes MW, Leonard RJ, Najarian JS, Michael AF, Brown DM. Pancreatic islet transplantation effects-on the glomerular lesions of experimental dibetes in rats. Diabetes, 1974; 23(9): 748-753.

19. Mauer MS, Steffes MW, Sutherland DER, Najarian JS, Michael AF, Brown DM. Studies of the rate of regression of the glomerular lesions in diabetic rats treated with pancreatic islet transplantation. Diabetes, 1975; 23(3): 280-285.

20. Merkovitch V, Campiche M. Intrasplenic autotransplantation of canine pancreatic tissue: maintenance of normoglycemia after total pancreatectomy. Eur Surg Res, 1977; 9: 173-190.

21. Kretschmer GJ, Sutherland DER, Matas AF, Steffes MW, Najarian JS. The dispersed pancreas: transplantation without islet purification in totally pancreatectomized dogs. Diabetologia, 1977; (13): 495-502.

22. Warnock GL, Rajotte RV, Procyshyn AW. Normoglycemia after reflux of islet containing fragments into the splenic vascular bed in dogs. Diabetes, 1983; 32(5): 452-458.

23. Reckard C, Barker C. Transplantation of isolated pancreatic islets across strong and weak histocompatibility barriers. Transplant Proc, 1973; 5(1): 761-763.

24. Reckard C, Zeigler M, Barker C. Physiologic and immunologic consequences of transplanting isolated pancreatic islets. Surgery, 1973; 74(1): 91-99.

25. Lacy PE, Davie JM, Finke EH. Prolongation of islet allograft survival following invitro culture (24°C) and a single inject of ALS. Science, 1979; 204: 312-313.

26. Lacy PE. Experimental immunoalteration. World J Surg, 1984; 8: 198-203.

27. Lafferty K, Cooley M, Woolnough J, Walker, K. Thyroid allograft immunogenecity is reduced after a period in organ culture. Science, 1975; 188: 259-261.

28. Lafferty K. The immunogenecity of foreign tissue. Transplantation, 1980; 29(3): 179-182.

29. Scharp DW. Isolation and transplantation of islet tissue. World J Surg. 1984; 8: 143-151.

30. Cahill GF, Arky RA. Diabetes. In: Rubenstein E, Federman D, eds. Scientific American Medicine. New York: Scientific American Inc., 1983; 9(6): 1-20.

31. Freinkel, H. On the etiology of diabetes mellitus. In: Rifkin H, Raskin P, eds. Diabetes Mellitus, Vol. 5. Maryland: Prentice- Hall, 1981: 1-6.

32. Albin J, Rifkin H. Etiologies of diabetes mellitus. Med Clin North Am, 1984; 66(6): 1209-1225.

33. Naji A, Silvers WK, Bartlett ST, Francfort J, Barker C. Immunologic factors in pathogenesis and treatment of human and animal diabetes. World J Surg, 1984; 8: 214-220.

34. Cahill GF. The future of diabetes. In: Rifkin H, Raskin P, eds. Diabetes Mellitus, Vol 5. Maryland: Prentice-Hall, 1981: 375-378.

35. Lief PD. Renal impairment in diabetes mellitus. In: Rifkin H, Raskin P, eds. Diabetes Mellitus, Vol 5. Maryland: Prentice-Hall, 1981: 265-268.

36. Henkind P, Walsh JB. Diabetic retinopathy. In: Rifkin H, Raskin P, eds. Diabetes Mellitus, Vol 5. Maryland: Prentice-Hall, 1981: 253-258.

37. Rifkin H, Ross H. Control of diabetes and longterm complications. In: Rifkin H, Raskin P, eds. Diabetes Mellitus, Vol 5. Maryland: Prentice-Hall, 1981: 233-240.

38. Foster DW. Diabetes mellitus. In: Petersdorf RC, Adams RD, Braunwald E, et al, eds. Harrison's Principles of Internal Medicine. New York: McGraw-Hill, 1983: 671-679.

39. Lee CS, Mauer MS, Brown DM, Sutherland DER, Michael AF, Najarian, JS. Renal transplantation in diabetes mellitus in rats. J Exp Med, 1974; 139: 793-800.

40. Engerman R, Bloodworth JMB, Nelson S. Relationship of microvascular disease in diabetes to metabolic control. Diabetes, 1977; 26(8): 760-769.

41. Gray BN, Watkins B. Prevention of vascular complications of diabetes by pancreatic islet cell transplantation. Arch Surg, 1976; 3: 254-257.

42. Pirart J. Diabetes mellitus and its degenerative complications; a prospective study of 4,400 patients observed between 1947 and 1973. Diabetes Care, 1978; 1(3): 168-188.

43. Keiding HR, Root HE, Marble A. Importance of control of diabetes in prevention of vascular complications. JAMA, 1952; 150: 964-969.

44. Steno Study Group. Effect of 6 months of strict metabolic control on eye and kidney function in insulin-dependent diabetics with background retinopathy. Lancet, 1982(Jan 16): 121-123.

45. Eschwege E, Job E, Guyot-Argenton C, Aubry JP, Tchobroutsky G. Delayed progression of diabetic retinopathy by divided insulin administration. A further follow-up. Diabetologia, 1979; 16: 13-15.

46. Miki E, Fukuda M, Kuzuyu t, Kosaka K, Nakao K. Relation of the course of retinopathy to control of diabetes, age and therapeutic agents in diabetic Japanese patients. Diabetes, 1968; 18(2): 773-780.

47. Gabbay K, Fluckigen R. Clinical significance of glycosylated hemoglobin. In: Rifkin H, Raskin P, eds. Diabetes Mellitus, Vol 5. Maryland: Prentice-Hall, 1981: 241-246.

48. Raskin P. Transplants, artificial pancreas and new pharmocological advances. In: Rifkin H, Raskin P, eds. Diabetes Millitus, Vol 5. Maryland: Prentice-Hall, 1981: 367-374.

49. Reemsta K, Weber CJ. Pancreas and pancreatic islet cell transplantation. In: Sabistan DC, ed. Davies-Christopher Textbook of Surgery. Philadelphia: WB Saunders Co., 1981: 537-542.

50. Stein W, Steer ML. The pancreas. In: Schwartz SI, ed. Principles of Surgery. New York: McGraw-Hill, 1984: 1345-1373.

51. Brown J, Mullen Y, Clark W, Molnar G, Heininger D. Importance of hepatic portal circulation for insulin action in streptozotocin-diabetic rats transplanted with foetal pancreas. J Clin Invest, 1979; 64: 1688-1694.

52. Federlin KF, Bretzel RG. The effects of islet transplantation on complications in experimental diabetes of the rat. World J Surg, 1984; 8: 169-178.

53. Weil R, Nozawa M, Koss M, Weber C, Reemsta K, McIntosh R. Pancreatic transplantation in diabetic rats: renal function, morphology, ultrastructure and immunohistology. Surgery, 1975; 78(2): 142-148.

54. Steffes MW, Brown DM, Basgen JM, Mauer MS. Amelioration of mesangial volume and surface alterations following islet transplantation in diabetic rats. Diabetes, 1980; (29): 509-515.

55. Hoffman L, Mandel TE, Carter WM, Koulmanda M, Martin FIR, Campbell DG, McMillan N. A comparison between islet transplantation and parental insulin in the control of diabetes and prevention of renal complications in mice. Metabolism, 1983; 32(5): 451-456.

56. Pozza G, Seccni A, Bosi E, Micossi P, Piatti PM, Monti LD, Cristallo M, Pontiroli AE, Gelet A, Dubernard JM, Traeger J. Artificial insulin delivery systems versus pancreas transplantation: effect on metabolic control. Transplant Proc, 1985; 17(1): 358-359.

57. Wellman KF, Volk BW. Historical Review. In: Volk BW, Wellman KF, eds. The Diabetic Pancreas. New York: Plenum Press, 1977: 1-14.

58. Downing R. Historical review of pancreatic islet transplantation. World J Surg, 1984; 8: 137-142.

59. Van Beek C. Leonid V. Sobolev 1876-1919. Diabetes, 1979; 7(3): 246-248.

60. Connoly JE. Pancreatic whole organ transplantation. Surg Clin North Am, 1978; 58(2): 383-390.

61. Jonasson O. Transplantation of the pancreas 1978. Transplant Proc, 1979; 16(1): 325-330.

62. Gleidman ML, Gold M, Whittaker J, Rifkin H, Soberman R, Freed S, Tellis V, Veith F. Pancreatic duct to ureter anastomosis for exocrine drainage in pancreatic transplantation. Am J Surg, 1973; 125: 245-252.

63. Groth C, Tyden G, Lundgren G, Wilczek H, Klintmalm G, Ost L, Gunnarsson R, Ostman J. Segmental pancreatic transplantation with enteric exocrine diversion. World J Surg, 1984; 8: 257-261.

64. Kyriakides G, Arora V, Lifton J, Nuttal F, Miller J. Porcince pancreatic transplants II. Allotransplantation of duct ligated segments. J Surg Res, 1976; 20: 461-466.

65. Groth CG, Lundgren G, Gunnarsson R, Arner P, Berg B, Ostman J. Segmental pancreatic transplantation with duct ligation or drainage to a Jejunal roux-en-y loop in nonuremic diabetic patients. Diabetes, 1980; 29(suppl1): 3-9.

66. Kyriakides GK, Rabinovitch A, Miniz D, Olson L, Rappaport F, Miller J. Long-term study of vascularized free-draining intraperitoneal pancreatic segmental allografts in beagle dogs. J Clin Invest, 1981; 67: 292-303.

67. Traeger J, Dubernard J, Touraine JL, Neyra P, Malik MC, Pelissard C, Ruitton A. Pancreatic transplantation in man: a new method of pancreas preparation and results in diabetes correction. Transplant Proc, 1979; 16(1): 331-335.

68. Dubernard J, Traeger J, Bosi E, Gelet A, Yafi S, Devonec M, Piatti PM, Chiesa R, Martin X, Monein-Long B, Jouraine J, Pozza G. Transplantation for the treatment of insulin-dependent diabetes: clinical experience with polymer obstructed pancreatic grafts using neoprene. World J Surg, 1984; 8: 262-266.

69. Sutherland DER. Pancreas and islet transplantation registry data. World J Surg, 1984; 8: 270-275.

70. Sutherland DER, Kendell D. Clinical pancreas and islet transplant registry report. Transplant Proc, 1985; 17(1): 307-311.

71. Bewick M, Miller B, Compton F, Gonzales-Carillo, Augoustis A, Eaton B. Canine pancreatic endocrine function after interruption of pancreatic exocrine drainage. Transplantation, 1983; 36(3): 246-251.

72. Liu T, Sutherland DER, Heil J, Dunning M, Najarian JS. Beneficial effects of establishing pancreatic duct drainage into a hollow organ (bladder, jejunum, or stomach) compared to free intraperitoneal drainage or duct injection. Transplant Proc, 1985; 17(1): 366-371.

73. Sutherland DER, Matas AJ, Najarian JS. Pancreatic islet cell transplantation. Surg Clin North Am, 1978; 58(2): 365-382.

74. Browning H, Resnick P. Homologous and heterologous transplantation of pancreatic tissue in normal and diabetic mice. Yale J Biol Med, 1951; 24: 141-152.

75. Moskalewski S. Isolation and culture of the islets of langerhans of the guinea pig. General and Comparative Endocrinology: 1965; 5: 342-353.

76. Scharp DW, Kemp BC, Knight MJ, Ballinger WF, Lacy PE. The use of ficoll in the preparation of viable islets of langerhans from the rat pancreas. Transplantation, 1973; 16(6): 686-689.

77. Finke EH, Lacy PE, Ono J. Use of reflected green light for specific identification of islets in vitro after collagenase isolation. Diabetes, 1979; 28: 612-613.

78. Rumpf KD, Lohlein D, Pichlmayr R. Multiple transplantations of islets of langerhans. Eur Surg Res, 1977; 9: 403-410.

79. Henriksson C, Bergmark J, Claes G. Metabolic response to isologous transplantation of small numbers of isolated islets of langerhans in the rat. Eur Surg Res, 1977; 9: 411-418.

80. Kemp CB, Knight MJ, Scharp DW, Ballinger WF, Lacy PE. Efect of tranplantation site on the results of pancreatic islet isografts in diabetic rats. Diabetologia, 1973; 9: 486-491.

81. Matas AJ, Payne WD, Grotting JC, Sutherland DER, Steffes MW, Hertel BF, Najarian JS. Portal versus systemic transplantation of dispersed neonatal pancreas. Transplantation, 1977; 24(5): 333-337.

82. Griffin RC, Scharp DW, Hartman BK, Ballinger WF, Lacy PE. A metabolic study of intrahepatic portal vein islet isografts. Diabetes, 1977; 26: 201-214.

83. Feldman SD, Hirsnberg GE, Dode G, Rallman M, Scharp DW, Ballinger WF, Lacy PE. Intrasplenic islet isografts. Surgery, 1977; 82(3): 386-394.

84. Yasunami Y, Lacy PE, Finke E. A new site for islet transplantation - a peritoneal-omental pouch. Transplantation, 1983; 36(2): 181-182.

85. Lenoard RJ, Lazarow A, Herge O. Pancreatic islet transplantation in the rat. Diabetes, 1973; 22: 413-418.

86. Matas AJ, Sutherland DER, Steffes MW, Najarian JS. Islet transplantation using neonatal rat pancreas: quantitative studies. J Surg Res, 1976; 20: 143-147.

87. Mullen Y, Clark WR, Kemp J, Molnar JG, Brown J. Reversal of experimental diabetes by foetal rat pancreas II. critical procedures for transplantation. Transplantation Proc, 1977; 9(1): 329-332.

88. Brown J, Clark WR, Molnar JG, Mullen YS. Foetal pancreas transplantation for reversal of streptozotocin induced diabetes in rats. Diabetes, 1976; 25: 56-65.

89. McEvoy R, Hegre O. Syngeneic transplantation of foetal rat pancreas. III. Effect of insulin treatment on the growth and differintiation of pancreatic implants after reversal of diabetes. Diabetes, 1979; 28: 141-146.

90. Zeigler M, Reckard C, Barker C. Longterm metabolic and immunologic considerations in transplantation of pancreatic islets. J Surg Res, 1974; 16: 575-581.

91. Mirkovitch V, Campiche M. Intrasplenic autotransplantation of canine pancreatic tissue: maintenance of normoclycemia after total pancreatectomy. Eur Surg Res, 1977; 9: 173-190.

92. Scharp DW, Downing R, Merrel RC, Greider M. Isolating the elusive islet. Diabetes, 1980; 29(suppl.1): 19-30.

93. Scharp DW, Murphy JJ, Newton WT, Ballinger WF, Lacy PE. Transplantation of islets of langerhans in diabetic rhesus monkeys. Surgery, 1975; 88(1): 100-105.

94. Sutherland DER, Steffes MW, Bauer GE, McManus D, Noe B, Najarian JS. Isolation of human and porcine islets of langerhans and islet transplantation in pigs. J Surg Res, 1974; 16: 102-111.

95. Horaguchi A, Merrel RC. Preparation of viable islet cells from dogs by a new method. Diabetes, 1981; 30: 455-458.

96. Mehigan DG, Zuidema GD, Cameron JL. Pancreatic islet transplantation in dogs: critical factors in technique. Am J Surg, 1981; 141: 208-212.

97. Kretschmer GJ, Sutherland DER, Matas AJ, Payne WD, Najarian JS. Autotransplantation of pancreatic fragments to the portal vein and spleen of totally pancreatectomized dogs. Ann Surg, 1978; 187(1): 79-86.

98. Kolb E, Ruckert R, Largardier F. Intraportal and intrasplenic autotransplantation of pancreatic islets in the dog. Eur Surg Res, 1977; 9: 419-426.

99. Alderson D, Farndon JR. The metabolic effects of islet transplantation in the diabetic dog. Transplant Proc, 1984; 16(3): 831-833.

100. Alderson D, Farndon JR, Albert KGM. Islet transplantation corrects disordered lipid metabolism of diabetes in dogs. Transplant Proc, 1985; 17(1): 388-390.

101. Rajotte RV, Warnock GL, Procyshyn DW, Wieczorek K. Intrasplenic isografts of canine pancreatic islets, metabolic study. Transplant Proc, 1984; 16(3): 834-837.

102. Mehigan DG, Bell NR, Zuidema GD, Eggleston JC, Cameron JL. Disseminated intravascular coagulation and portal hypertension following pancreatic islet autotransplantation. Ann Surg 1980; 191(3): 287-293.

103. Miller BHR, Bewick M, Compton FJ, Needham JM, Godwin KL, Winter M. Disseminated intravascular coagulation after dispersed pancreas transplantation in dogs: causitive agents. Transplantation, 1983; 36(3): 348-350.

104. Lorenz D, Lippert H, Tietz W, Worm V, Hann NJ, Dorn A, Koch G, Ziegler M, Rosenbaum KD. Transplantation of isolated islets of langerhans in diabetic dogs: I. results after allogenic intraportal islet transplantation. J Surg Res, 1979; 27: 181-192.

105. Toledo-Peyera LH, Bandlein KO, Gordon DA, MacKenzie GH, Reyman TA. Renal subcapsular islet cell transplantation. Diabetes, 1984; 33: 910-914.

106. The Canadian Multicentre Transplant Study Group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. New Engl J Med, 1983; 309(14): 809-815.

107. Gunnarsson R, Klintmalm S, Lundgran G, Tyden S, Wilczek H, Ostman J, Groth CG. Deterioration in glucose metabolism in pancreatic transplant recipients after conversion from azothioprine to cyclosporin. Transplant Proc, 1984; 16(3): 709-712.

108. Reemsta K. Experimental islet cell grafting: a transplantation model. Trans Proc, 1970; 2(4): 513-515.

109. Nash JR, Peters M, Bell PRF. Comparable survival of pancreatic islets, heart, kidney and skin allografts in rats with and without enhancement. Transplantation, 1977; 24(1): 70-73.

110. Perloff LJ, Naji A, Silvers WK, McKearn JJ, Barker CF. Vascularized pancreas versus isolated islet allografts: an immunological comparison. Surgery, 1980; 88(2): 222-230.

111. Reckard CR, Stuart FP, Clayman JL, Buckingham F, Schulak JA. Differential susceptibility of segmental and isolated islet allografts to rejection and enhancement. Transplant Proc, 1981; 13(1): 819-822.

112. Finch DRA, Morris PJ. The effect of increasing islet numbers on survival of pancreatic islet allografts in immunosuppressed diabetic rats. Transplantation, 1977; 23(1): 104-106.

113. Lacy PE, Finke E, Janney C, Davie J. Prolongation of islet xenograft survival by invitro culture of rat megaislets in 95% O_2. Transplantation, 1982; 33(6): 588-592.

114. Lacy PE, Davie JM, Finke EH. Effect of culture on islet rejection. Diabetes, 1980; 29(suppl/.1): 93-97.

115. Bowen K, Andrus L, Lafferty K. Successful allotransplantation of mouse pancreatic islets to nonimmunosuppressed recipients. Diabetes, 1980; 29: 98-103.

116. Yasunami Y, Lacy PE, Davie JM, Finke E. Prolongation of islet xenograft survival (rat to mouse) by invitro culture at 37°C. Transplantation, 1983; 33(4): 281-284.

117. Faustman D, Lacy PE, Davie JM. Transplantation without immunosuppression. Diabetes, 1982; 3(suppl.4): 11-13.

118. Naji A, Silvers WK, Barker CF. Influence of organ culture on the survival of major histocompatibility complex compatible and incompatible parathyroid allografts in rats. Transplantation, 1981; 32(4): 296-298.

119. Lacy PE, Davie J, Finke E. Induction of rejection of successful allografts of rat islets by donor peritoneal exudate cells. Transplantation, 1979; 28(5): 415-420.

120. Parr E. The absence of H_2 antigens from mouse pancreatic β cells demonstrated by immunoferritin labelling. J Exp Med, 1979; 150: 1-9.

121. Hart DNJ, Newton MR, Reece-Smith H, Fabre JW, Morris PJ. Major histomcompatibility complex antigens in the rat pancreas, isolated pancreatic islets, thyroid and adrenal. Transplantation, 1983; 36(4): 431-435.

122. Naji A, Silvers WK, Barker CF. Transplantation, 1983; 36(4): 355-361.

123. Weber C, Ting W, Rosenkrantz K, Rivera S, Pernis B, Reemsta K. Islet transplantation in diabetes aladbies mellitus. 1984; 16(3): 845-848.

124. Squifflet SP, Sutherland DER, Florack G, Morrow CE, Najarian JS. Physiologic comparison of segmental pancreas and islet transplants in rats. Transplant Proc, 1985; 17(1): 378-380.

125. Uhlschmid G, Largiader F. Pancreas organ preservation: a review of the literature and results of the authors. Transplant Proc, 1980; 12 (No.4,Suppl.2): 157-162.

126. Rajotte RV, Warnock GL, Bruch LC, Procyshyn AW. Transplantation of cryopreserved and fresh rat islets and canine pancreactic fragments: comparison of cryopreservation protocols. Cryobiology, 1983; 20: 169-184.

127. Rajotte RV, Warnock GL, Kneteman NM. Cryopreservation of insulin-producing tissue in rats and dogs. World J Surg, 1984; 8: 179-186.

128. Izumi R, Konishi K, Shimizuk, Hirosawa H, Takahashi N, Miyazaki I. Isolation of human pancreatic islets from cryopreserved pancreas. Transplant Proc, 1985; 17(1): 383-384.

129. Walsh TJ, Eggleston JC, Cameron JL. Portal hypertension, hepatic infarction and liver failure complicating pancreatic islet autotransplantation. Surgery, 1982; 91(4): 485-487.

130. Long JA, Britt L, Olack BJ, Scharp DW. Autotransplantation of isolated canine islets. Transplant Proc, 1983; 15(1): 1332-1337.

131. Lacy PE, Lacy ET, Finke EH, Yasunami Y. An improved method for the isolation of islets from the beef pancreas. Diabetes, 1982; 31(suppl.4): 109-111.

132. Downing R, Scharp DW, Ballinger WF. An improved technique for isolation and identification of mammalian islets of langerhans. Transplantation, 1980; 29(29): 79-83.

133. Ono J, Takaki R, Fukuma M. Preparation of single cells from pancreatic islets of the adult rat by the use of dispase. Endocrinol Japan, 1977; 24(3): 265-270.

134. Henrikkson C, Bergmark J, Claes G. Use of trypsin for isolation of islets of langerhans in the rat. Eur Surg Res, 1977; 9: 427-431.

135. Hinshaw DB, Jolley WB, Hinshaw DB, Kaiser JE, Hinshaw K. Islet autotransplantation after pancreatectomy for pancreatitis with a new method of islet preparation. Am J Surg, 1981; 142: 118-122.

136. Hasiguchi H, Taguchi T, Takeda Y, Ursell P, Klomp GF, Dobell WH. Whole-organ incubation as a first step in the isolation of pancreatic islets from large animals. Artif organs, 1983; 7(2): 250-253.

137. Gray DWR, McShane P, Morris PJ. A method for isolation of islets of langerhans from the human pancreas. Transplant Proc, 1985; 17(1): 381-382.

138. Payne WD, Sutherland DER, Matas AJ, Gorecki P, Najarian JS. DL-ethionine treatment of adult pancreatic donors. Amelioration of diabetes in multiple recipients with tissue from a single donor. Ann Surg, 1979; 189(2): 148-256.

139. Vrbova H, Theodorov NA, Tyhurst M, Howell SL. Transplantation of islets of langerhans from pilocarine-pretreated rats. A method of enhancing islet yield. Transplantation, 1979; 28(5): 433-435.

140. Lazarow A, Wells LJ, Carpenter AM, Hegre O, Leonard RJ, McEvoy RC. Islet differentiation, organ culture, and transplantation. Diabetes, 1973; 22(1): 877-912.

141. Matas AJ, Sutherland DER, Steffes MW, Najarian JS. Short-term culture of adult pancreatic fragments for purification and transplantation of islets of langerhans. Surgery, 1976; 80(2): 183-191.

142. Matas AJ, Sutherland DER, Kretschmer G, Steffes MW, Najarian JS. Pancreatic tissue culture: depletion of exocrine enzymes and purification of islets for transplantation. Transplant Proc, 1977; 9(1): 337-339.

143. Lundgren G, Andersson A, Borg H, Buschard K, Groth CG, Gunnarrson R, Hellerstrom C, Ostman J. Structural and functional integrity of isolated human islets of langerhans maintained in tissue culture for 1-3 weeks. Transplant Proc, 1977; 9(1): 237-240.

144. Andersson A, Borg H, Groth CG, Gunarrson R, Hellerstrom C, Lundgren G, Westman J, Ostman J. Survival of isolated human islets of langerhans maintained in tissue culture. J Clin Invist, 1976; 57: 1295-1301.

145. Weber CJ, Hardy MA, Lerner RL, Reemsta K. Tissue culture isolation and preservation of human cadaveric pancreatic islets. Surgery, 1977; 81(3): 270-273.

146. Reemsta K, Weber CJ, Pi-Sunyer FX, Lerner RL, Hardy MA. Alternatives in pancreatic transplantation. Tissue culture studies. Diabetes, 1980; 29(suppl.1): 45-51.

147. Merrel RC, Schap DW, Gingerich R, Feldmeier M, Greider M, Downing R. New approaches to separation of islet cells. Surgical Forum, 1979; 30: 303-304.

148. Britt LD, Stojeba PC, Scharp CR, Greider MH, Scharp DW. Neonatal pig pseudoislets. A product of selective aggregation. Diabetes, 1981; 30: 580-583.

149. Lindhall PE. Principle of a counter-streaming centrifuge for the separation of particles of different sizes. Nature, 1948; 164: 648-649.

150. Faustman DL, Steinman RN, Gebel HM, Hauptfeld V, Davie JM, Lacy PE. Prevention of mouse islet allograft rejection by elimination of intraislet dendritic cells. Transplant Proc, 1985; 17(1): 420-422.

151. Hardy MA, Lau HT, Reemsta K. Prolongation of rat islet allografts with the use of ultraviolet irradiation, without immunosuppression. Transplant Proc, 1984; 16(3): 865-869.

152. Hardy MA, Lau HT, Weber C, Reemsta K. Pancreatic islet transplantation: immunoalteration with ultraviolet irradiation. World J Surg, 1984; 8: 207-213.

153. Mullen Y, Yoneda K, Fujiya H, Brown J, Sasaki N, Danilous A. Survival of cultured foetal pancreas transplants in miniature pigs. Transplant Proc, 1985; 17(1): 417-419.

154. Faustman D, Lacy P, Davie J, Hauptfeld V. Prevention of allograft rejection by immunization with donor blood depleted of Ia-bearing cells. Science, 1982; 217: 157-158.

155. Lau H, Reemsta K, Hardy MA. Pancreatic islet allograft prolongation by donor-specific blood transfusions treated with ultraviolet radiation. Science, 1983; 221: 754-755.

156. Serie JR, Hickey GE, Schmitt RV, Hegre OD. Prolongation of cultured-isolated neonatal islet xenografts without immunosuppression. Transplantation, 1983; 36(1): 6-11.

157. Bobzien B, Yasunami Y, Majercik M, Lacy PE, Davie JM. Intratesticular transplants of islet xenografts (rat to mouse). Diabetes, 1983; 32: 213-216.

158. Toledo-Pereya LH, Bandlien KO, Gordon DA, MacKenzie GH, Reyman TA. Renal subcapsular islet cell transplantation. Diabetes, 1984; 33: 910-914.

159. Robbins SL, Cotran RS. Pathologic basis of disease .2nd ed. Philadelphia: WB Saunders Co., 1979: 550-558.

160. Ritter MA. Radiobiology of mammalian cells. Semin Oncolog, 1981; 8(1): 3-17.

161. Little JB, Williams JR. Effects of ionizing radiation in mammalian cells. In: Lee DH, ed: Handbook of Physiology. Bethesda: American Physiologic Society, 1977; (9): 127.

162. Casarett GW: Radiation histopathology. Boca Raton FL: CRC Press, 1980; (1): 1-49.

163. Duncan W, Nias AHW. Clinical radiobiology. Edinburgh: Churchill Liuchgstone, 1977.

164. Andrews HL. Radiation biophysics. New Jersey. Prentice-Hall, 1974.

165. Berdjis CC. Pathology of irradiation. Baltimore: Wiliams and Williams Co., 1977: 1-31.

166. Chapman DJ. Hypoxic sensitizors - implications for radiation therapy. N Engl J Med, 1979; 301(26): 1429-1432.

167. Fajardo LF, Berthrong M. Radiation injury in surgical pathology. Part III. salivary glands, pancreas and skin. Am J Surg Path, 1981; 5(3): 279-296.

168. Casarett GW. Radiation histopathology. Boca Raton, FL: CRC Press, 1980; (2): 58-61.

169. White DC. An atlas of radiation histopathology. Virginia: National Technical Information Service, 1975: 93-105.

170. Sommers SC. Effects of ionizing radiation upon endocrine glands. In: Berdjis CC, ed. Pathology of Irradiation. Baltimore: Williams and Wilkins, 1971: 434-435.

171. Fisher HF, Groot JT, Bachem A. The effects of x-ray on the pancreas. Am J Physiol, 1926; 76: 299-305.

172. Leven NL. An experimental study: the effects of radium emanation on the pancreas of dogs. Am J Cancer, 1933; 18: 899-904.

8

173. Volk BW, Wellman KF, Lewitan A. The effects of radiation on the fine structure and enzymes of the dog pancreas. I short-term studies. Am J Pathol, 1966; 48: 721-753.

174. Archembeau J, Griem M, Harper P. The effects of 250-kv x-rays on the dogs pancreas: morphological and functional changes. Radiation Research, 1966; 28: 243-256.

175. Rauch RF, Stentstrom KW. Effects of x-ray radiation on pancreatic function in dogs. Gastroenterology, 1952; 20(4): 595-603.

176. Wellman KF, Volk BW, Lewitan A. The effect of radiation on the fine structure and enzyme content of the dog pancreas. II. Long-term studies. Lab Invest, 1966; 15: 1007-1023.

177. Pieroni PO, Rudick J, Adler M, Nacchiero M, Rybak BJ, Perlberg JH, Dreiling DA. Effect of irradiation on the canine exocrine pancreas. Ann Surg, 1976; 184: 610-614.

178. Devonec M, Faure JL, Blanc-Bruhai H, Dubernard JM, Traeger J. Effects of pancreatic intraductal injection of a radioisotope in dogs. Transplant Proc, 1980; 12(4 suppl.2): 141-144.

179. Faure JL, Provensal B, Dubernard JM, Martin X, Devonec M, Margonari J, Traeger J. Effects of intraductal irradiation n the canine pancreas. Transplant Proc, 1984; 16(3): 752-754.

180. Zook BC, Bradley EW, Casarett GW, Rogers CC. Pathologic effects of fractionated fast neutrons or photons on the pancreas, pylorus and duodenum of dogs. Int J Radiation Oncology Biol Phys, 1983; 9(10): 1493-1504.

181. Spaulding JF, Lushbaugh CC. Radiopathology of islets of laugerhans in rats (Abstract). Fed Proc, 1955; 14: 420.

182. Sejanov GC, Gulgaev VA. the death of A-cells of the pancreatic langerhans' islets following irradiation. Radiobiol-Radiother, 1983: 24(4): 453-458.

183. Merkel FK, Kelly WD, Goetz FG, Maney J. Irradiated heterotopic segmental canine pancreatic allografts. Surgery, 1968; 63(2): 291-297.

184. Kyriakides G, Miller J, Lifton J, Nuttal F. Exocrine function in duct ligated porcine pancreatic autografts (Abstract). Diabetes, 1974; 23: 359.

185. Kyriakides GK, Vijender K, Arora K, Lifton J, Nuttal FQ, Miller J. Porcine pancreatic transplantation I: autotransplantation of duct ligated segments. J Surg Res, 1976; 20(5): 451-460.

186. Tersigni R, Toledo-Peyera LH, Palestini M, Cavallini M, Capua G, Caramla F, Stipa S. Effect of ex vivo hypothermic irradiation on duct-ligated pancreas allografts in dogs: functional, histological and ultrastructural findings. American Surgeon, 1980; 46: 713-721.

187. Kelly WD, Lillehi RC, Merkel FK, Idezuki Y, Goetz FC. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery, 1967; 61(6): 827-837.

188. Tersigni R, Toledo-Peyera LH, Falluca F, Cavallini M, Capece G, Giagrande L, Capua G, Stipa S. Transplantation of irradiated heterotoric segmental human pancreas. American Surgeon, 1983; 49: 502-505.

189. Lacy PE, Davi JM, Finke EH, Scharp DW. Prolongation of islet allograft survival. Transplantion, 1979; 27(3): 171-174.

190. Lacy PE, Walker MM, Finke CJ. Perifusion of isolated rat islets invitro-participation of the microtubular system in the biphasic release of insulin. Diabetes, 1972; 21(10): 987-991.

191. Lacy PE, Finke EH, Conant S, Naber S. Long-term perifusion of isolated rat islets invitro. Diabetes, 1976; 25(4): 484-493.

192. Cobb LF, Merrel RC. Total pancreatectomy in dogs. J Surg Res, 1984; 37(3): 235-240.

193. Watson ML. Staining of tissue sections for electron microscopy with heavy metals. J Biophys Biochem Cytol, 1958; 4: 475-478.

194. Reynolds ES. The use of lead citrate at high ph as an electron-opaque stain in electron microscopy. J Cell Biol, 1963; 17: 208-212.

195. Morgan CR, Lazarow A. Immunoassay of insulin: two-antibody system. Diabetes, 1983; 12: 115.

196. Pierre KG, Tung KK, Nadj H. A new enzymatic kinetic method for determination of a-amylase (Abstract). Clin Chem, 1976; 22: 1219.

197. Kadish AH. A new method for the continuous monitoring of blood glucose by measurement of dissolved oxygen. Clin Chem, 1965; 11: 869.

198. Moorehouse JA, Grahame GR, Rosen NJ. Relationship between intravenous glucose tolerance and the fasting blood glucose level in healthy and in diabetic subjects. J Clin Endocrinol, 1964; 24: 145-159.

199. Swift H, Hruban Z. Focal degradiation as a biological process. Fed Proc, 1964; 23: 1026-1037.

200. Kneteman NM, Rajotte RV, Procyshyn AW. Canine pancreatic fragment allotransplantation with cyclosporine A. Arch Surg (in press).